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BIOCOMPATIBLE CROSSLINKED POLYMERS

The present patent application is a continuation in part of U.S. Patent Application

No. 09/147,897, entitled "Methods And Devices For Preparing Protein Concentrates"

10   which is a United States national stage application of Patent Cooperation Treaty

application PCT/US/16897 (publication number WO 98/12274), which has a priority date

based on United States applications 60/026,526; 60/039,904; and 60/040,417. The present

patent application is also a continuation-in-part of U.S. Patent Application No.

09/454,900, entitled "Biocompatible Crosslinked Polymers" which has a priority date

15   based on United States patent application 60/110,849. The present patent application

claims priority to these other patents and patent applications which are hereby

incorporated by reference herein.

Field Of The Invention

20   The present invention relates generally to biocompatible crosslinked polymers,

methods for preparing and using same.

Background of the Invention

Almost every surgical treatment carries a risk that bodily tissues exposed during

25   the course of the surgery will adhere to each other, a condition termed an adhesion.

Gynecological and abdominal surgeries, in particular, are prone to causing adhesions,

which often have the appearance of scar-like masses. Adhesions are frequently painful

5   and are a significant cause of infertility resulting from gynecological surgeries. Adhesions caused by surgeries are often called surgical adhesions.

One approach to the treatment of adhesions has been to coat surgically exposed tissues with a gel before closing the surgical site. Gels of various types have been used, including suspensions of colloidal particles, and pastes of natural polymers. Various

10   examples of some of these approaches are described in U.S. Patent Nos. 6,020,326 and 5,605,938. Some of these approaches allow for the polymers to be added to the patient "in situ" in a solution and then chemically reacted inside the patient so that the polymers form covalent crosslinks to create a polymer network. This approach lets the polymer be formed in a way that closely conforms to the shape of the tissues in the body, as

15   described, for example, in United States patents 5,410,016; 5,573,934 and 5,626,863.

Hydrogels are especially useful for use in the body because they are more biocompatible than non-hydrogels and are thus better tolerated in the body. Besides being useful for post-operative adhesions they can be used for many medical purposes, such as tissue augmentation, medical device coating, surgical sealing, and drug delivery.

20   Examples of hydrogels formulated for such purposes are found in U.S. patents 4,414,976; 4,427,651; 4,925,677; 5,527,856; 5,550,188; and 5,814,621.

Crosslinked polymers have previously been formed using polymers equipped with either electrophilic or nucleophilic functional groups. For example, U.S. Patent Nos. 5,296,518 and 5,104,909 to Grasel et al. describe the formation of crosslinked polymers

25   from ethylene oxide rich prepolymers, wherein a polyisocyanate or low molecular weight diisocyanate is used as the electrophilic polymer or crosslinker, and a polyoxyethylene

5 based polyol with in situ generated amine groups is used as the nucleophilic precursor; see also U.S. patents 5,514,379; 5,527,856; and 5,550,188.

Polymeric hydrogels, for example, fibrin glue, crosslinked proteins, and crosslinked polyethylene oxides, are essentially colorless. This problem is often even more acute when the hydrogel is applied as a coating on a tissue because tissue coatings 10 conventionally are thin. The resulting colorless solution or film is therefore difficult to visualize, especially in the typically wet and moist surgical environment. Under laparoscopic conditions, visibility is even more difficult due to the fact that only a two-dimensional view of the surgical field is available on the monitor that is used in such procedures.

Summary of the Invention

The present inventors have realized that use of color in biocompatible crosslinked polymers and precursors greatly improves their performance in a surgical environment, especially under minimally invasive surgical procedures (MIS), e.g., laparoscopic, endoscopic. Moreover, the better visibility available with the use of color also permits 20 efficient use of materials and avoids wastage.

An embodiment of the invention is a hydrogel for use on a substrate such as a patient's tissue. The hydrogel has water, a biocompatible visualization agent, and reactive hydrophilic polymers that form a crosslinked hydrogel after contact with the tissue. The hydrogel coats the tissue and forms a coating. The coating may have a free 25 surface. The visualization agent is disposed in the hydrogel and reflects or emits light at a wavelength detectable to a human eye. This feature lets a user applying the hydrogel

5 observe the hydrogel and estimate its thickness and apply the hydrogel until it reaches a predetermined thickness.

The hydrophilic polymers may be natural polymers, for example proteins e.g., collagen, fibrinogen, albumin, and fibrin, polysaccharides, or glycosaminoglycans,. The polymers can also have a hydrolytically biodegradable portion and/or a proteolytically

10 degradable portion. The polymers are preferably covalently crosslinked and are crosslinkable via an electrophilic functional group-nucleophilic functional group reaction. An embodiment of the invention is a hydrogel that is coated onto a tissue and has a maximum thickness of between 0.1 to 10.0 mm.

Preferred biocompatible visualization agents are FD&C Blue #1, #2, #3 , D&C

15 Green #6, and methylene blue. The visualization agent may also be a fluorescent molecule. The visualization agent is preferably not covalently linked to the hydrogel.

Methods for using the polymeric compositions to coat a tissue include mixing hydrophilic precursor polymers with chemically distinct reactive functional groups such that they form crosslinks via nucleophilic-electrophilic reaction after mixing and contact

20 with the tissue. The polymers crosslink to form a biodegradable hydrogel. A preferred application is to prevent surgical adhesions by applying the hydrogel as a coating on a tissue substrate and maintaining another surface of the hydrogel as a free surface. A visualization agent is preferably included so that the visualization agent is disposed within the hydrogel and reflects or emits light at a wavelength detectable to a human eye.

25 A preferred method of use is to form a hydrogel on the tissue until the color and/or color intensity of the hydrogel indicates that a predetermined thickness of hydrogel has been deposited on the tissue.

5   An embodiment of the invention is a polymeric product made by a process of mixing hydrophilic polymers having nucleophilic functional groups with hydrophilic polymers having electrophilic functional groups such that they form a mix that crosslinks after contact with the tissue of a patient to form a biodegradable hydrogel that coats a tissue. In many applications it is desirable to also have a free surface. The hydrogel

10   preferably contains a visualization agent in the mix of reactive precursor species so that the visualization agent is disposed within the interior and reflects or emits light at a wavelength detectable to a human eye.

An embodiment of the invention is a kit having a biocompatible visualization agent, at least two chemically distinct reactive precursor species, and instructions for

15   using the visualization agent and the reactive precursor species such that the reactive precursor species may be combined to form crosslinked hydrophilic polymers that form a biodegradable hydrogel. In another embodiment, the visualization agent is premixed with one of the reactive precursor species.

It is an object of the present invention to provide biocompatible crosslinked

20   polymers and methods for their preparation and use, in which the biocompatible crosslinked polymers are formed without using free radical chemistry, and are formed using at least one non-toxic small molecule precursor.

It is another object of this invention to provide such biocompatible crosslinked polymers and methods for their preparation and use, in which the biocompatible

25   crosslinked polymers are formed from aqueous solutions, preferably under physiological conditions.

5   It is still another object of this invention to provide such biocompatible crosslinked polymers and methods for their preparation and use, in which the biocompatible crosslinked polymers are formed in vivo.

It is a still further object of this invention to provide such biocompatible crosslinked polymers and methods for their preparation and use, in which the

10   biocompatible crosslinked polymers are biodegradable.

Another object of this invention is to provide such biocompatible crosslinked polymers and methods for their preparation and use, in which the biocompatible crosslinked polymers, their precursors, or both are colored.

Another object of this invention is to provide methods for preparing tissue

15   conforming, biocompatible crosslinked polymers in a desirable form, size and shape.

Another object of this invention is to provide methods for using biocompatible crosslinked polymers to form medically useful devices or implants for use as surgical adhesion prevention barriers, as implantable wound dressings, as scaffolds for cellular growth for tissue engineering or as surgical tissue adhesives or sealants.

20   Another object of this invention is to provide methods for using biocompatible crosslinked polymers to form medically useful devices or implants that can release bioactive compounds in a controlled manner for local, systemic, or targeted drug delivery.

Another object of this invention is to provide methods and compositions for

25   producing composite biomaterials comprising fibers or particulates made of biodegradable biocompatible crosslinked polymers.

5   Brief Description of the Drawings

FIG. 1 depicts electrophilic functional group water soluble and biodegradable crosslinkers or functional polymers, which can be crosslinked with appropriate nucleophilic functional group precursors.

10   FIG. 2 depicts nucleophilic water soluble and biodegradable crosslinkers or functional polymers, which can be crosslinked with appropriate electrophilic precursors.

FIG. 3 depicts electrophilic water soluble and biodegradable crosslinkers or functional polymers, which can be crosslinked with appropriate nucleophilic functional group precursors, wherein either the biodegradable linkages or the functional groups are 15 selected so as to make the precursor water soluble.

FIG. 4 depicts nucleophilic functional group water soluble crosslinkers or functional polymers, which can be crosslinked with appropriate electrophilic functional group precursors, and which are not biodegradable.

FIG. 5 depicts electrophilic water soluble crosslinkers or functional polymers, 20 which can be crosslinked with appropriate nucleophilic functional group precursors, and which are not biodegradable.

FIG. 6 depicts the preparation of an electrophilic water soluble crosslinker or functional polymer using carbodiimide ("CDI") activation chemistry, its crosslinking reaction with a nucleophilic water soluble functional polymer to form a biocompatible 25 crosslinked polymer product, and the hydrolysis of that biocompatible crosslinked polymer to yield water soluble fragments.

5   FIG. 7 depicts the use of sulfonyl chloride activation chemistry to prepare an electrophilic functional polymer.

FIG. 8 depicts the preparation of an electrophilic water soluble crosslinker or functional polymer using N-hydroxysuccinimide ("NHS") activation chemistry, its crosslinking reaction with a nucleophilic water soluble functional polymer to form a

10   biocompatible crosslinked polymer product, and the hydrolysis of that biocompatible crosslinked polymer to yield water soluble fragments.

FIG. 9 depicts preferred NHS esters for use in the invention.

FIG. 10 shows the N-hydroxysulfosuccinimide ("SNHS") activation of a tetrafunctional sugar-based water soluble synthetic crosslinker and its crosslinking

15   reaction with 4-arm amine terminated polyethylene glycol to form a biocompatible crosslinked polymer product, and the hydrolysis of that biocompatible crosslinked polymer to yield water soluble fragments.

FIG. 11 shows the variation in gelation time with the number of amino groups for the reaction of 4 arm 10 kDa succinimidyl glutarate PEG ("SG-PEG") with di-,tri- or

20   tetra-lysine.

FIG. 12 shows the variation in gelation time with the solution age of the electrophilic functional polymer.

FIG. 13 shows the variation in gelation time with the concentration of biocompatible crosslinked polymer precursors, and with the solution age of the 4 arm 10

25   kDa carboxymethyl - hydroxybutyrate -N- hydroxysuccinimidyl PEG ("CM-HBA-NS") electrophilic functional polymer.

5   FIG. 14 shows the variation in degradation time with the concentration of

biocompatible crosslinked polymer.

Detailed Description of the Invention

The present inventors have realized that use of color in biocompatible crosslinked

10   polymers and/or reactive precursor species improves the performance of crosslinked

networks of polymers and/or reactive precursor species in a surgical environment,

especially for minimally invasive surgical (MIS) procedures. Many applications have the

best results when an appropriate or predetermined amount of hydrogel is delivered to the

surgical environment, for example when applied to the surface of a substrate such as a

15   tissue. A hydrogel that is too thick may reduce efficiency or interfere with other surgical

aspects. For example, if a hydrogel is applied too thickly, it could interfere with closure

of the wound or interfere with tissue movement, e.g., in intestinal applications. A

hydrogel that is too thin will not serve its purpose, e.g., providing a barrier that prevents

surgical adhesions or provides a strong seal against fluid leakage. The introduction of a

20   visualization agent allows the user to determine the thickness of the applied hydrogel.

The visualization agent is preferably an agent that provides a color that is visible to the

human eye, e.g., a color that is detected visually by the user or detected by a video

camera and relayed to a video screen observed by the user.

Conventional polymeric hydrogels may sometimes have a faint inherent color or

25   develop a faint color as a result of chemical activity, but their lack of color makes a layer

of a hydrogel very difficult to see after it has been applied to a tissue. Hydrogels have

sometimes been mixed with image contrast agents to increase their visibility for medical

5   imaging devices such as X-ray or magnetic resonance imaging (MRI) machines, as in, for

example, U.S. patent 5,514,379. Colorants have also been used for hydrogels injected

into bodily tissues, for example in U.S. patents 5,514,379 and 6,124,273.

The use of a visualization agent is especially preferred when a hydrogel is used to

coat a substrate. A substrate coating surface is a surface of a hydrogel that contacts a

10   substrate and, in the region of contact, is essentially in continuous contact with that

substrate. Although some small portions of the coating or substrate may not be in

contact, the contact is intimate. A substrate coating surface can be formed when the

hydrogel crosslinks after contacting the substrate surface because the contact before

crosslinking allows the hydrogel precursors to mix and conform to the shape of the

15   substrate. A preformed hydrogel material generally does not have a substrate coating

surface. A preferred substrate is a tissue of a patient.

A hydrogel with a substrate coating surface preferably also has a free surface

when the hydrogel is used for prevention of adhesions. The hydrogel is applied to a

tissue and crosslinks while having one free surface that is not adherent to any tissue but is

20   instead freely movable relative to any tissues that it may subsequently contact. The free

surface prevents the coated tissue from contact with other tissues and does not prevent the

movement of other tissues so that protection and free movement are optimal. In this

situation, a user that applies the hydrogel may observe the hydrogel by looking through

the free surface into the hydrogel and at the coated tissue. A visualization agent in the

25   hydrogel makes the hydrogel change in its appearance until the user determines that the

thickness of the hydrogel is sufficient. For example, a blue dye in the hydrogel makes the

hydrogel increasingly opaque as the thickness of the hydrogel increases.

5   It is preferable to provide color by adding a colored visualization agent to the hydrogel precursors before crosslinking. The coloring agent is thus present in a premixed amount that is already selected for the application. A preferred embodiment of the invention uses biocompatible crosslinked polymers formed from the reaction of precursors having electrophilic functional group and nucleophilic functional groups. The

10   precursors are preferably water soluble, non-toxic and biologically acceptable.

Preferably, at least one of the precursors is a small molecule of about 1000 Da or less, and is referred to as a "crosslinker". The crosslinker preferably has a solubility of at least 1 g/100 mL in an aqueous solution. A crosslinked molecule may be crosslinked via an ionic or covalent bond, a physical force, or other attraction. Preferably, at least one of

15   the other precursors is a macromolecule, and is referred to as a "functional polymer". The macromolecule, when reacted in combination with a crosslinker, is preferably at least five to fifty times greater in molecular weight than the small molecule crosslinker and is preferably less than about 60,000 Da. A more preferred range is a macromolecule that is seven to thirty times greater in molecular weight than the crosslinker and a most preferred

20   range is about ten to twenty times difference in weight. Further, a macromolecular molecular weight of 5,000 to 50,000 is preferred, a molecular weight of 7,000 to 40,000 is more preferred and a molecular weight of 10,000 to 20,000 is most preferred. The term polymer, as used herein, means a molecule formed of at least three repeating groups. The term "reactive precursor species" means a polymer, functional polymer,

25   macromolecule, small molecule, or crosslinker that can take part in a reaction to form a network of crosslinked molecules, e.g., a hydrogel.

5   An embodiment of the invention is a hydrogel for use on a patient's tissue that has water, a biocompatible visualization agent, and crosslinked hydrophilic polymers that form a hydrogel after contact with the tissue. The hydrogel coats the tissue and also has a free surface. The visualization agent reflects or emits light at a wavelength detectable to a human eye so that a user applying the hydrogel can observe the gel and also estimate its

10   thickness.

         Natural polymers, for example proteins or glycosaminoglycans, e.g., collagen, fibrinogen, albumin, and fibrin, may be crosslinked using reactive precursor species with electrophilic functional groups. Natural polymers are proteolytically degraded by proteases present in the body. Synthetic polymers and reactive precursor species are

15   preferred, however, and may have electrophilic functional groups that are carbodiimidazole, sulfonyl chloride, chlorocarbonates, n-hydroxysuccinimidyl ester, succinimidyl ester or sulfasuccinimidyl esters. The term synthetic means a molecule that is not found in nature, e.g., polyethylene glycol. The nucleophilic functional groups may be, for example, amine, hydroxyl, carboxyl, and thiol. The polymers preferably have a

20   polyalkylene glycol portion. More preferably they are polyethylene glycol based. The polymers preferably also have a hydrolytically biodegradable portion or linkage, for example an ester, carbonate, or an amide linkage. Several such linkages are well known in the art and originate from alpha-hydroxy acids, their cyclic dimmers, or other chemical species used to synthesize biodegradable articles, such as, glycolide, dl-lactide, l-lactide,

25   caprolactone, dioxanone, trimethylene carbonate or a copolymer thereof. A preferred embodiment has reactive precursor species with two to ten nucleophilic functional groups

5   each and reactive precursor species with two to ten electrophilic functional groups each. The hydrophilic species are preferably synthetic molecules.

Preferred biocompatible visualization agents are FD&C BLUE #1, FD&C BLUE #2, and methylene blue. These agents are preferably present in the final electrophilic-nucleophilic reactive precursor species mix at a concentration of more than 0.05 mg/ml

10   and preferably in a concentration range of at least 0.1 to about 12 mg/ml, and more preferably in the range of 0.1 to 4.0 mg/ml, although greater concentrations may potentially be used, up to the limit of solubility of the visualization agent. These concentration ranges were found to give a color to the hydrogel that was desirable without interfering with crosslinking times (as measured by the time for the reactive

15   precursor species to gel). The visualization agent may also be a fluorescent molecule. The visualization agent is preferably not covalently linked to the hydrogel.

An embodiment of the invention is a hydrogel that is coated onto a tissue and generally has at least a portion with a thickness of between 0.8 to 12.0 mm. One technique for measuring the thickness is to create a hydrogel on a test surface and use a

20   micrometer to measure thicknesses at various points. Alternatively, a calibrated videomicroscopic image could be used. The preferred thickness depends on the medical application but a preferred thickness for prevention of surgical adhesions is about 0.5 to 10.0 mm, and more preferably about 0.8 to 5 mm and even more preferably about 1 -3 mm.

25   A preferred method of use is to form a hydrogel on the tissue until the color of the hydrogel indicates that a predetermined thickness of hydrogel has been deposited on the tissue. The deposition of the precursors that result in formation of the hydrogel may be

5   by spraying, dripping, or delivery via a catheter. The user may apply the hydrogel to a test surface with a color that resembles the surface that the user contemplates using and observe the color that results when the hydrogel reaches a desired thickness that the user has predetermined. In use, the user applies the hydrogel until the desired color is reached. A typical patient's tissue has a pinkish appearance and the microvasculature can

10   be observed as thin lines. One embodiment is to introduce a concentration of visualization agent into the hydrogel so that the user applies the hydrogel until the microvasculature is no longer visible through the hydrogel, at which point the hydrogel is a desired thickness. Another suitable method is to apply the hydrogel until the underlying tissue is obscured. An appropriately selected concentration of visualization

15   agent is used so that the hydrogel obscures the tissue features when the hydrogel achieves a predetermined thickness. The predetermined thickness is chosen to correspond to the particular application. In these thickness evaluation approaches, a concentration that is too low will result in a hydrogel that is too thick and a concentration that is too high will result in a hydrogel that is too thin. Thus, the visualization agent allows the user to

20   ascertain the presence of the hydrogel on the surface and also gain feedback on the appropriate thickness, preferably in combination with instructions provided as part of a kit. In some embodiments, suitable approaches can be used with visualization agents and polymers that crosslink by, for example, free radical polymerization, electrophilic functional group-nucleophilic functional group interaction.

25   An embodiment of the invention is a method of a user applying a hydrogel coating to a substrate and selecting a visually observable visualization agent to observe the hydrogel coating. The user may use visualization agents to see the hydrogel with the

5   human eye or with the aid of an imaging device that detects visually observable

visualization agents, e.g., a videocamera. A visually observable visualization agent is an

agent that has a color detectable by a human eye. A characteristic of providing imaging

to an X-ray or MRI machine is not a characteristic sufficient to establish function as a

visually observable visualization agent. An alternative embodiment is a visualization

10   agent that may not normally be seem by the human eye but is detectable at a different

wavelength, e.g., the infra red or ultraviolet, when used in combination with a suitable

imaging device, e.g., a videocamera.

A coating has a surface that can be viewed for use with a visually observable

visualization agent. In contrast, a hydrogel injected into a blood vessel, muscle, or other

15   tissue has essentially no surface for viewing a visualization agent because its surface area

is essentially engaged with tissues of the patient. Further, polymers injected into a tissue

lack a surface that is disposed on the surface of a tissue and do not provide a means for a

user to control the thickness of the coating on the surface of the tissue. Hydrogels that

are merely injected into a patient's body would not be equivalent to embodiments of the

20   present invention that involve a hydrogel coating on a substrate and are inoperative for

embodiments of the invention that entail use of a visualization agent in a hydrogel

coating.

An embodiment of the invention involves a mixture or a process of mixing

hydrophilic reactive precursor species having nucleophilic functional groups with

25   hydrophilic reactive precursor species having electrophilic functional groups such that

they form a mixture that crosslinks quickly after contact with the tissue of a patient to

form a biodegradable hydrogel that coats and adheres to a tissue. This may be achieved

5   by making reactive precursor species that crosslink quickly after mixing. Hydrophilic

reactive precursor species can be dissolved in buffered water such that they provide low

viscosity solutions that readily mix and flow when contacting the tissue. As they flow

across the tissue, they conform to the shape of the small features of the tissue such as

bumps, crevices and any deviation from molecular smoothness. If the reactive precursor

10   species are too slow to crosslink, they will flow off the tissue and away into other

portions of the body with the result that the user will be unable to localize the hydrogel

on the desired tissue. Without limiting the invention to a particular theory of operation, it

is believed that reactive precursor species s that crosslink appropriately quickly after

contacting a tissue surface will form a three dimensional structure that is mechanically

15   interlocked with the coated tissue. This interlocking contributes to adherence, intimate

contact, and essentially continuous coverage of the coated region of the tissue.

Adherence is important for medical applications that require a coating, e.g., for

prevention of adhesions, since a user must be able to place the hydrogel in the portions of

the patient that are needful, for example, around an ovary or surrounding an intestine.

20   Further, the hydrogel must remain on the intended tissue or it will be unable to provide a

prophylactic barrier. The hydrogels of the invention have good adhesion onto tissue and

are useful for all applications wherein surgical glues have previously been used. For

example, sealing of the dura mater of the brain to prevent leakage of cerebrospinal fluid

may be accomplished with combinations of reactive precursor species described herein

25   by using reactive precursor species with nucleophilic functional groups for mixing with

hydrophilic reactive precursor species having electrophilic functional groups to form a

5   mix that crosslinks quickly after contact with the tissue of a patient, e.g., the dura mater, to form a hydrogel that coats a tissue.

A simple dip test that shows that a hydrogel has adherence. To perform this test, a gel of about 5 x 5 centimeters in length x width and about 4 to 10 mm in thickness is formed on a substrate, the hydrogel is immersed in water or physiological saline for five

10   minutes, removed, and tilted to an angle of 90 degrees above horizontal, and dipped into and out of a vessel of physiological saline five times at a rate of about 10 mm per second so that the hydrogel passes through the air-water interface ten times. Then the substrate is rotated about 90 degrees so that the substrate is approximately horizontal and the hydrogel is below the substrate. The substrate is left in this position for five minutes.

15   The gel passes the dip test if less than about 1 square centimeter of the gel is then observed to be separated from the substrate. If the substrate lacks stiffness, it may be affixed to a stiff support so that it may tested. Physiological saline, in the context of the dip test, means a saline solution with an approximately physiological osmolarity and a pH of 7.0-7.4 at room temperature that is customarily used in cell culture, for example,

20   phosphate buffered saline. As used herein, the gel has adherence to a substrate if it passes the dip test.

Suitable crosslinking times vary for different applications. In most applications, the crosslinking reaction leading to gelation occurs within about 10 minutes, more preferably within about 2 minutes, even more preferably within 10 seconds. In the case

25   of most surgical adhesion prevention applications, it is preferable to use a hydrogel that crosslinks in less than about 10 seconds and more preferably in about 2- 4 seconds in order to allow a user to make multiple passes with a hydrogel applicator tool such as a

5 sprayer; see, for example commonly assigned U.S. patents 6,179,862; 6,165,201; 6,152,943; and U.S. patent applications 09/687,588, which are hereby incorporated herein by reference. In the case of tissues that can be accessed only indirectly, longer times are most preferable to allow the gel a longer time to flow into the inaccessible space. For example, application of an adhesion barrier in and around the spinal cord and exiting

10 nerve roots following spine surgery may require several extra seconds to penetrate around the complex geometry of the tissues so that a preferred time is between about 5 and about 90 seconds and more preferably between about 10 and about 30 seconds. The Examples herein describe a variety of reactive precursor species and methods of making reactive precursor species that may be mixed to provide crosslinked networks that crosslink

15 quickly after mixing such that one skilled in these arts will understand how to make the materials of the invention after reading this disclosure.

Functional Groups

 Each precursor is multifunctional, meaning that it comprises two or more

20 electrophilic or nucleophilic functional groups, such that a nucleophilic functional group on one precursor may react with an electrophilic functional group on another precursor to form a covalent bond. At least one of the precursors comprises more than two functional groups, so that, as a result of electrophilic-nucleophilic reactions, the precursors combine to form crosslinked polymeric products. Such reactions are referred to as "crosslinking

25 reactions".

 Preferably, each precursor comprises only nucleophilic or only electrophilic functional groups, so long as both nucleophilic and electrophilic precursors are used in the crosslinking reaction. Thus, for example, if a crosslinker has nucleophilic functional

5   groups such as amines, the functional polymer may have electrophilic functional groups

such as N-hydroxysuccinimides. On the other hand, if a crosslinker has electrophilic

functional groups such as sulfosuccinimides, then the functional polymer may have

nucleophilic functional groups such as amines or thiols. Thus, functional polymers such

as proteins, poly(allyl amine), or amine-terminated di-or multifunctional poly(ethylene

10   glycol) ("PEG") can be used.

Water Soluble Cores

The precursors preferably have biologically inert and water soluble cores. When

the core is a polymeric region that is water soluble, preferred polymers that may be used

include: polyether, for example, polyalkylene oxides such as polyethylene

15   glycol("PEG"), polyethylene oxide ("PEO"), polyethylene oxide-co-polypropylene oxide

("PPO"), co-polyethylene oxide block or random copolymers, and polyvinyl alcohol

("PVA"); poly (vinyl pyrrolidinone) ("PVP"); poly (amino acids); dextran and proteins

such as albumin. The polyethers and more particularly poly(oxyalkylenes) or

poly(ethylene glycol)or polyethylene glycol are especially preferred. When the core is

20   small molecular in nature, any of a variety of hydrophilic functionalities can be used to

make the precursor water soluble. For example, functional groups like hydroxyl, amine,

sulfonate and carboxylate, which are water soluble, maybe used to make the precursor

water soluble. In addition, N-hydroxysuccinimide ("NHS") ester of subaric acid is

insoluble in water, but by adding a sulfonate group to the succinimide ring, the NHS ester

25   of subaric acid may be made water soluble, without affecting its reactivity towards amine

groups.

Biodegradable Linkages

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5   If it is desired that the biocompatible crosslinked polymer be biodegradable or absorbable, one or more precursors having biodegradable linkages present in between the functional groups may be used. The biodegradable linkage optionally also may serve as the water soluble core of one or more of the precursors. In the alternative, or in addition, the functional groups of the precursors may be chosen such that the product of the

10   reaction between them results in a biodegradable linkage. For each approach, biodegradable linkages may be chosen such that the resulting biodegradable biocompatible crosslinked polymer will degrade or be absorbed in a desired period of time. Preferably, biodegradable linkages are selected that degrade under physiological conditions into non-toxic products.

15   The biodegradable linkage may be chemically or enzymatically hydrolyzable or absorbable. Illustrative chemically hydrolyzable biodegradable linkages include polymers, copolymers and oligomers of glycolide, dl-lactide, 1-lactide, caprolactone, dioxanone, and trimethylene carbonate. Illustrative enzymatically5 hydrolyzable biodegradable linkages include peptidic linkages cleavable by metalloproteinases and

20   collagenases. Additional illustrative biodegradable linkages include polymers and copolymers of poly(hydroxy acid)s, poly(orthocarbonate)s, poly(anhydride)s, poly(lactone)s, poly(aminoacid)s, poly(carbonate)s, and poly(phosphonate)s.

Visualization Agents

        Where convenient, the biocompatible crosslinked polymer or precursor solutions

25   (or both) may contain visualization agents to improve their visibility during surgical procedures. Visualization agents are especially useful when used in MIS procedures, due among other reasons to their improved visibility on a color monitor.

5   Visualization agents may be selected from among any of the various non-toxic colored substances suitable for use in medical implantable medical devices, such as FD&C BLUE dyes 3 and 6, eosin, methylene blue, indocyanine green, or colored dyes normally found in synthetic surgical sutures. The preferred color is green or blue because it has better visibility in presence of blood or on a pink or white tissue background. Red is

10   the least preferred color, when used on a highly vascularized tissue that is red in color. However, red may be suitable when the underlying tissue is white, for example the cornea.

The visualization agent may be present with either reactive precursor species, e.g.., a crosslinker or functional polymer solution. The preferred colored substance may

15   or may not become chemically bound to the hydrogel.

The visualization agent may be used in small quantities, preferably less than 1% weight/volume, more preferably less that 0.01% weight/volume and most preferably less than 0.001% weight/volume concentration.

Additional visualization agents may be used, such as fluorescent (e.g., green or

20   yellow fluorescent under visible light) compounds (e.g., fluorescein or eosin), x-ray contrast agents (e.g., iodinated compounds) for visibility under x-ray imaging equipment, ultrasonic contrast agents, or MRI contrast agents (e.g., Gadolinium containing compounds).

Visually observable visualization agents are preferred. Wavelengths of light from

25   about 400 to 750 nm are observable to the human as colors (R.K. Hobbie, Intermediate Physics for Medicine and Biology, 2nd Ed., pages 371-373). Blue color is perceived when the eye receives light that is predominantly from about 450 to 500 nm in

5   wavelength and green is perceived at about 500 to 570 nm (Id.). The color of an object

is therefore determined by the predominant wavelength of light that it reflects or emits.

Further, since the eye detects red or green or blue, a combination of these colors may be

used to simulate any other color merely by causing the eye to receive the proportion of

red, green, and blue that is perceived as the desired color by the human eye. Blue and

10   green visualization agents are preferred since they are most readily visible when

observing in situ crosslinking due to the approximately red color of the background color

of tissue and blood. The color blue, as used herein, means the color that is perceived by a

normal human eye stimulated by a wavelength of about 450 to 500 nm and the color

green, as used herein, means the color that is perceived by a normal human eye

15   stimulated by a wavelength of about 500 to 570 nm.

Crosslinking Reactions

The crosslinking reactions preferably occur in aqueous solution under

physiological conditions. More preferably the crosslinking reactions occur "in situ",

meaning they occur at local sites such as on organs or tissues in a living animal or human

20   body. More preferably the crosslinking reactions do not release heat of polymerization.

Preferably the crosslinking reaction leading to gelation occurs within about 10 minutes,

more preferably within about 2 minutes, more preferably within about one minute, and

most preferably within about 30 seconds. When it is desirable to build up a coating on a

convex surface, the crosslinking reaction preferably occurs within about 2 minutes, more

25   preferably in 30-60 seconds, and most preferably in 2-4 seconds.

Certain functional groups, such as alcohols or carboxylic acids, do not normally

react with other functional groups, such as amines, under physiological conditions (e.g.,

5   pH 7.2-11.0, 37°C). However, such functional groups can be made more reactive by using an activating group such as N-hydroxysuccinimide. Several methods for activating such functional groups are known in the art. Preferred activating groups include carbonyldiimidazole, sulfonyl chloride, aryl halides, sulfosuccinimidyl esters, N-hydroxysuccinimidyl ester, succinimidyl ester, epoxide, aldehyde, maleimides,

10   imidoesters and the like. The N-hydroxysuccinimide esters or N-hydroxysulfosuccinimide groups are the most preferred groups for crosslinking of proteins or amine functionalized polymers such as amino terminated polyethylene glycol ("APEG").

FIGS. 1 to 5 illustrate various embodiments of preferred crosslinkers and

15   functional polymers.

FIG. 1 illustrates possible configurations of degradable electrophilic crosslinkers or functional polymers. The biodegradable regions are represented by (^^^^^^^) ; the functional groups are represented by (◀) and the inert water soluble cores are represented by (——). For crosslinkers, the central core is a water soluble small

20   molecule and for functional polymers the central core is a water soluble polymer of natural or synthetic origin.

When Structure A in FIG. 1 is a functional polymer, it is a linear water soluble and biodegradable functional polymer, end-capped with two functional groups (e.g., N-hydroxysuccinimide ester or NHS, epoxide or similar reactive groups). The water soluble

25   core may be a polyalkylene oxide, preferably polyethylene glycol block copolymer, and it is extended with at least one biodegradable linkage between it and each terminal

5 functional group. The biodegradable linkage may be a single linkage or copolymers or homopolymers of absorbable polymers such as polyhydroxy acids or polylactones.

When Structure B in FIG. 1 is a functional polymer it is a branched or star shaped biodegradable functional polymer which has an inert polymer at the center. Its inert and water soluble core is terminated with oligomeric biodegradable extensions, which in turn

10 are terminated with reactive functional groups.

When Structures C and D in FIG. 1 are functional polymers, they are multifunctional 4 arm biodegradable functional polymers. This polymer again has a water-soluble soluble core at the center, which is a 4 arm, tetrafunctional polyethylene glycol (Structure C) or block copolymer of PEO-PPO-PEO such as Tetronic 908

15 (Structure D) which is extended with by small oligomeric extensions of biodegradable polymer to maintain water solubility and terminated with reactive functional end-groups such as CDI or NHS.

When Structure E in FIG. 1 is a functional polymer, it is a multifunctional star or graft type biodegradable polymer. This polymer has a water-soluble polymer like

20 polyethylene oxide, polyvinyl alcohol or poly(vinyl pyrrolidinone) at the core which is completely or partially extended with biodegradable polymer. The biodegradable polymer is terminated with reactive end groups.

Structures A-E in FIG. 1 need not have polymeric cores and may be small molecule crosslinkers. In that case, the core may comprise a small molecule like

25 ethoxylated glycerol, inositol, trimethylolpropane etc. to form the resultant crosslinker. In addition, Structures A-E in FIG. 1 need not have polymeric biodegradable extensions, and the biodegradable extensions may consist of small molecules like succinate or

5 glutarate or combinations of 2 or more esters, such as glycolate/2-hydroxybutyrate or

glycolate/4-hydroxyproline, etc. A dimer or trimer of 4-hydroxyproline may be used not

only to add degradability, but also to add nucleophilic functional group reactive sites via

the pendant primary amines which are part of the hydroxyproline moiety.

Other variations of the core, the biodegradable linkage, and the terminal

10 electrophilic group in Structures A-E in FIG. 1 may be constructed, so long as the

resulting functional polymer has the properties of low tissue toxicity, water solubility,

and reactivity with nucleophilic functional groups.

FIG. 2 illustrates various embodiments of nucleophilic biodegradable water

soluble crosslinkers and functional polymers suitable for use with electrophilic functional

15 polymers and crosslinkers described herein.

The biodegradable regions are represented by (^^^^^^^); the functional groups

are represented by (·\\ |); and the inert water soluble cores are represented by (——).

For crosslinkers, the central core is a water soluble small molecule and for functional

polymers the central core is a water soluble polymer of natural or synthetic origin.

20

When Structure F in FIG. 2 is a functional polymer, it is a linear water soluble

biodegradable polymer terminated with reactive functional groups like primary amine.

The linear water-soluble core is a polyalkylene oxide, preferably polyethylene glycol

block copolymer, which is extended with the biodegradable region which is a copolymer

25 or homopolymers of polyhydroxy acids or polylactones. This biodegradable polymer is

terminated with primary amines.

5   When Structure G in FIG. 2 is a functional polymer, it is a branched or star shaped biodegradable polymer which has an inert polymer at the center. The inert polymer is extended with single or oligomeric biodegradable extensions which are terminated with reactive functional groups.

When Structures H and I in FIG. 2 are functional polymers, they are

10   multifunctional 4 arm biodegradable polymers. These polymers again have water-soluble cores at their center which are either a 4 arm, tetrafunctional polyethylene glycol (Structure H) or a block copolymer of PEO-PPO-PEO such as Tetronic 908 (Structure I), extended with small oligomeric extensions of biodegradable polymers to maintain water solubility, and terminated with functional groups such as amines and thiols.

15   When Structure J in FIG. 2 is a functional polymer, it is a multifunctional star or graft type biodegradable polymer. This polymer has a water soluble polymer like polyethylene oxide, polyvinyl alcohol or poly(vinyl pyrrolidinone) at the core which is completely or partially extended with biodegradable polymer. The biodegradable polymer is terminated with reactive end groups.

20   Structures F-J in FIG. 2 need not have polymeric cores and may be small molecule crosslinkers. In that case, the core may comprise a small molecule like ethoxylated glycerol, inositol, trimethylolpropane etc. to form the resultant crosslinker.

Other variations of the core, the biodegradable linkage, and the terminal nucleophilic functional group in Structures F-J in FIG. 2 may be constructed, so long as

25   the resulting functional polymer has the properties of low tissue toxicity, water solubility, and reactivity with electrophilic functional groups.

5 FIG. 3 illustrates configurations of water-soluble electrophilic crosslinkers or functional polymers where the core is biodegradable. The biodegradable regions are represented by (^^^^^^^) and the functional groups are represented by (◀) . The biodegradable core is terminated with a reactive functional group that is also water solubilizing, such a N-hydroxysulfosuccinimide ester ("SNHS") or N-

10 hydroxyethoxylated succinimide ester ("ENHS").

Structure K in FIG. 3 depicts a difunctional biodegradable polymer or oligomer terminated with SNHS or ENHS. The oligomers and polymers may be made of a poly(hydroxy acid) such as poly(lactic acid), which is insoluble in water. However, the terminal carboxylic acid group of these oligomers or polymers can be activated with N-

15 hydroxysulfosuccinimide ester ("SNHS") or N-hydroxyethoxylated succinimide ester ("ENHS") groups. An ionic group, like a metal salt (preferably sodium salt) of sulfonic acid, or a nonionic group, like a polyethylene oxide on the succinimide ring, provides water-solubility while the NHS ester provides chemical reactivity towards amines. The sulfonate groups (sodium salts) or ethoxylated groups on the succinimide ring solubilize

20 the oligomer or polymer without appreciably inhibiting reactivity towards amine groups.

Structures L-0 in FIG. 3 represent multi-branched or graft type structures with terminal SNHS or ENHS group. The cores may comprise various non-toxic polyhydroxy compounds like sugars (xylitol, erythritol), glycerol, trimethylolpropane, which have been reacted with anhydrides such as succinic or glutaric anhydrides. The resultant acid

25 groups were then activated with SNHS or ENHS groups to form water soluble crosslinkers or functional polymers.

5   FIG. 4 illustrates various nucleophilic functional polymers or crosslinkers that are not biodegradable. The nucleophilic functional groups are represented by (⦀) and the inert water-soluble cores are represented by (———). For crosslinkers, the central core is a water-soluble small molecule and for functional polymers the central core is a water soluble polymer of natural or synthetic origin.

10   When Structure P in FIG. 4 is a functional polymer it may be a water-soluble linear polymer such as polyethylene glycol terminated with reactive end group such as primary amines and thiols. Such polymers are commercially available from Sigma (Milwaukee, WI) and Shearwater Polymers (Huntsville, AL). Some other preferred difunctional polymers are PPO-PEO-PPO block copolymers such as Pluronic F68

15   terminated with amine groups. Pluronic or Tetronic polymers are normally available with terminal hydroxyl groups. The hydroxyl groups are converted into amine groups by methods known in the art.

       When Structures Q-T in FIG. 4 are functional polymers they may be multifunctional graft or branch type water soluble copolymers with terminal amine

20   groups.

       Structures P-T in FIG. 4 need not have polymeric cores and may be small molecule crosslinkers. In that case, the core may comprise a small molecule like ethoxylated glycerol, inositol, trimethylolpropane, dilysine etc. to form the resultant crosslinker.

25   Other variations of the core and the terminal nucleophilic functional group in Structure P-T in FIG. 4 may be employed, so long as the properties of low tissue toxicity, water solubility, and reactivity with electrophilic functional groups are maintained.

5   FIG. 5 illustrates various electrophilic functional polymers or crosslinkers that are

not biodegradable. The electrophilic functional groups are represented by (◀) and the

inert water soluble cores are represented by (———). For crosslinkers, the central core is a

water soluble small molecule and for functional polymers the central core is a water

soluble polymer of natural or synthetic origin.

10   When Structure U is a functional polymer, it may be a water-soluble polymer

such as polyethylene glycol terminated reactive end group such as NHS or epoxide. Such

polymers are commercially available from Sigma and Shearwater polymers. Some other

preferred polymers are PPO-PEO-PPO block copolymers such as Pluronic F68

terminated with NHS or SNHS group. Pluronic or Tetronic polymers are normally

15   available with terminal hydroxyl groups. The hydroxyl groups are converted into acid

group by reacting with succinic anhydride. The terminated acid groups are reacted with

N-hydroxysuccinimide in presence of DCC to generate NHS activated Pluronic polymer.

         When Structures V-Y are functional polymers they may be multifunctional graft

or branch type PEO or PEO block copolymers (Tetronics) activated with terminal

20   reactive groups such as NHS.

         Structures U-Y in FIG. 5 need not have polymeric cores and may be small

molecule crosslinkers. In that case, the core may comprise a small molecule like

ethoxylated glycerol, tetraglycerol, hexaglycerol, inositol, trimethylolpropane, dilysine

etc. to form the resultant crosslinker.

25   Other variations of the core and the terminal nucleophilic functional group in

Structures U-Y in FIG. 5 may be employed, so long as the properties of low tissue

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5 toxicity, water solubility, and reactivity with electrophilic functional groups are maintained.

<u>Preparation of Structures A-Y in FIGS. 1-5</u>

The polymeric crosslinkers and functional polymers illustrated as Structures A-Y in FIGS. 1 to 5 may be prepared using variety of synthetic methods. Their preferred

10 compositions are described in Table 1.

Table 1. Preferred Crosslinkers and Functional Polymers

Structure	Brief Description	Typical Example
A	Water soluble, linear difunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences which are cleavable by enzymes and terminated with protein reactive functional groups	Polyethylene glycol or ethoxylated propylene glycol chain extended with oligolactate and terminated with N-hydroxysuccinimide esters
B	Water soluble, trifuncational crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	Ethoxylated glycerol chain extended with oligolactate and terminated with N-hydroxysuccinimide esters
C	Water soluble, tetrafunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	4 arm polyethylene glycol, erythritol or pentaerythritol or pentaerythritol chain extended with oligolactate and terminated with N-hydroxysuccinimide esters
D	Water soluble, tetrafunctional	Ethoxylated ethylene diamine or

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	crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like Tetronic 908 chain extended with oligotrimethylene carbonate and terminated with N-hydroxysuccinimide ester
E	Water soluble, branched crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	Low molecular weight polyvinyl alcohol with 1% to 20% hydroxyl groups extended with oligolactate and terminated with N-hydroxysuccinimide ester
F	Water soluble, liner difunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer surfactant like Pluronic F68 chain extended with oligolactate and terminated with amino acids such as lysine or peptide sequences that may contain two amine groups
G	Water soluble, trifunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Ethoxylated glycerol chain extended with oligolactate and terminated with aminoacid such as lysine
H	Water soluble, tetrafuncational crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	4 arm polyethylene glycol or tetra erythritol chain extended with oligolactate and terminated with aminoacid such as lysine
I	Water soluble, tetrafunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of	Ethoxylated ethylene diamine or polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like Tetronic 908 chain extended with oligotrimethylene

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	hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	carbonate and terminated with aminoacid such as lysine
J	Water soluble, multifunctional or graft type crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Low molecular weight polyvinyl alcohol with 1-20% hydroxyl groups extended with oligolactate and terminated with aminoacid such as lysine
K	Water soluble, linear difunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Difunctional oligolactic acid with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimi de ester or ethoxylated n-hydroxysuccinimide ester.
L	Water soluble branched trifunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Trifunctional oligocaprolactone with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimi de ester or ethoxylated n-hydroxysuccinimide ester.
M	Water soluble, branched tetrafunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Tetrafunctional oligocaprolactone with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimi de ester or ethoxylated n-hydroxysuccinimide ester.
N	Water soluble, branched tetrafunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Tetrafunctional oligocaprolatone with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimi de ester or ethoxylated n-hydroxysuccinimide ester.
O	Water soluble, branched multifunctional crosslinker or functional polymer such as oligomers f hydroxyacids or peptide sequences which are	Multifunctional oligolactic acid with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimi de ester or ethoxylated n-hydroxysuccinimide

	terminated with protein reactive functional groups	ester.
P	Water soluble, linear difunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols functional groups	Polyethylene glycol with terminal amines groups
Q	Water soluble, branched trifunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols as functional group	Ethoxylated glycerol with terminal amines groups.
R	Water soluble, branched tetrafunctional crosslinker of functional polymer terminated with amines, carboxylic acid or thiols functional groups	4 arm polyethylene glycol modified to produce terminal amine groups
S	Water soluble, branched tetrafunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols functional groups	Ethoxylated ethylene diamine or polyethylene oxide-polyprophylene oxide-polyethylene oxide block copolymer like Tetronic 908 modified to generate terminal amine groups
T	Water soluble, branched or graft crosslinker or functional polymer with terminal amines, carboxylic acid or thiols functional groups	Polylysine, albumin, polyallyl amine
U	Water soluble, linear difunctional crosslinker or functional polymer terminated with protein reactive functional groups	Polylysine, albumin, polyallyl amine
V	Water soluble branched trifunctional crosslinker or functional polymer terminated with protein reactive functional groups	Ethoxylated glycerol terminated with n-hydroxysuccinimide
W	Water soluble branched tetrafunctional crosslinker or functional polymer terminated with protein reactive functional groups	4 arm polyethylene glycol terminated with n-hydroxysuccinimide esters
X	Water soluble branched tetrafunctional crosslinker or functional polymer terminated with protein reactive functional	Ethoxylated ethylene diamine or polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like Tetronic 908 with n-

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	groups	hydroxysuccinimide ester as end group
Y	Water soluble, branched or graft polymer crosslinker or functional polymer with protein reactive functional groups	Poly (vinyl pyrrolidinone)-co-poly (n-hydroxysuccinimide acrylate) copolymer (9:1), molecular weight < 40000 Da

First, the biodegradable links of Structures A-J in FIGS. 1 and 2 may be composed of specific di or multifunctional synthetic amino acid sequences which are recognized and cleaved by enzymes such as collagenase, and may be synthesized using methods known to those skilled in the peptide synthesis art. For example, Structures A-E in FIG. 1 may be obtained by first using carboxyl, amine or hydroxy terminated polyethylene glycol as a starting material for building a suitable peptide sequence. The terminal end of the peptide sequence is converted into a carboxylic acid by reacting succinic anhydride with an appropriate amino acid. The acid group generated is converted to an NHS ester by reaction with N-hydroxysuccinimide.

The functional polymers described in FIG. 2 may be prepared using a variety of synthetic methods. In a preferred embodiment, the polymer shown as Structure F may be obtained by ring opening polymerization of cyclic lactones or carbonates initiated by a dihydroxy compound such as Pluronic F 68 in the presence of a suitable catalyst such as stannous 2-ethylhexanoate. The molar equivalent ratio of caprolactone to Pluronic is kept below 10 to obtain a low molecular weight chain extension product so as to maintain water solubility. The terminal hydroxyl groups of the resultant copolymer are converted into amine or thiol by methods known in the art.

In a preferred method, the hydroxyl groups of a Pluronic-caprolactone copolymer are activated using tresyl chloride. The activated groups are then reacted with lysine to

5   produce lysine terminated Pluronic-caprolactone copolymer. Alternatively, an amine-blocked lysine derivative is reacted with the hydroxyl groups of a Pluronic-caprolactone copolymer and then the amine groups are regenerated using a suitable deblocking reaction.

Structures G, H, I and J in FIG. 2 may represent multifunctional branched or graft 10 type copolymers having water soluble core extended with oligohydroxy acid polymer and terminated with amine or thiol groups.

For example, in a preferred embodiment, the functional polymer illustrated as Structure G in FIG. 2 is obtained by ring opening polymerization of cyclic lactones or carbonates initiated by a tetrahydroxy compound such as 4 arm, tetrahydroxy 15 polyethylene glycol (molecular weight 10,000 Da), in the presence of a suitable catalyst such as stannous octoate. The molar equivalent ratio of cyclic lactone or carbonate to PEG is kept below 10 to obtain a low molecular weight extension, and to maintain water solubility (polymers of cyclic lactones generally are not as water soluble as PEG). Alternatively, hydroxyacid as a biodegradable link may be attached to the PEG chain 20 using blocking/deblocking chemistry known in the peptide synthesis art. The terminal hydroxy groups of the resultant copolymer are activated using a variety of reactive groups known in the art. The CDI activation chemistry and sulfonyl chloride activation chemistry is shown in FIGS. 6 and 7, respectively.

The most preferred reactive groups are N-hydroxysuccinimide esters, synthesized 25 by any of several methods. In a preferred method, hydroxyl groups are converted to carboxylic groups by reacting them with anhydrides such as succinic anhydride in the presence of tertiary amines such as pyridine or triethylamine or dimethylaminopyridine

5   ("DMAP"). Other anhydrides such as glutaric anhydride, phthalic anhydride, maleic anhydride and the like may also be used. The resultant terminal carboxyl groups are reacted with N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide ("DCC") to produce N-hydroxysuccinimide ester (referred as NHS activation). The NHS activation and crosslinking reaction scheme is shown in FIG. 8. The most preferred N-

10 hydroxysuccinimide esters are shown in FIG. 9.

In a preferred embodiment, the polymer shown as structure H is obtained by ring opening polymerization of glycolide or trimethylene carbonate initiated by a tetrahydroxy compound such as tetrafunctional polyethylene glycol (molecular weight 2000 Da) in the presence of a catalyst such as stannous 2-ethylhexoate. The molar equivalent ratio of

15 glycolide to PEG is kept from 2 to 10 to obtain a low molecular weight extension. The terminal hydroxy groups of the resultant copolymer are converted into amine groups by reaction with lysine as mentioned previously. Similar embodiments can be obtained using analogous chain extension synthetic strategies to obtain structures F, G, I and J by starting with the appropriate corresponding polyol.

20 Structures K, L, M, N and O in FIG. 3 are made using a variety of synthetic methods. In a preferred embodiment, the polymer shown as Structure L in FIG. 3 is obtained by ring opening polymerization of cyclic lactones by a trihydroxy compound such as glycerol in the presence of a catalyst such as stannous 2-ethylhexanoate. The molar equivalent ratio of cyclic lactone to glycerol is kept below 2, so that only low

25 molecular weight oligomers are obtained. The low molecular weight oligomer ester is insoluble in water. The terminal hydroxy groups of the resultant copolymer are activated using N-hydroxysulfosuccinimide groups. This is achieved by converting hydroxy groups

5   to carboxylic groups by reacting with anhydrides such as succinic anhydride in presence

of tertiary amines. The resultant terminal carboxyl groups are reacted with N-

hydroxysulfosuccinimide or N-hydroxyethoxylated succinimide in the presence of

dicyclohexylcarbodiimide ("DCC") to produce a sulfonated or ethoxylated NHS ester.

The sulfonate or PEO chain on the succinimide ring gives water solubility to the

10   oligoester.

The foregoing method generally is applied to solubilize only low molecular

weight multi-branched oligoesters, with molecular weights below 1000. In another

variation of this method, various non-toxic polyhydroxy compounds, preferably sugars,

such as erythritol, xylitol are reacted with succinic anhydride in the presence of a tertiary

15   amine. The terminal carboxyl group of succinated erythritol is esterified with N-

hydroxysulfosuccinimide (FIG. 9). Similar embodiments may be obtained using

analogous synthetic strategies to obtain structures K, and M-O by starting with the

appropriate starting materials.

Structures P-R may be synthesized by reacting the appropriate starting material,

20   such as a linear (P) or 2- or 3-arm branched PEG (Q, R) with hydroxy end groups, with

lysine as mentioned previously, such that the arms of the PEG oligomers are capped with

amine end groups. Structure S may be synthesized, using a multistep reaction, from PEG,

glycerol and a diisocyanate. In the first step a PEG diol is reacted with excess

diisocyanate, such as 4, 4'diphenyl methane diisocyanate ("MDI"), methylene-bis (4-

25   cyclohexylisocyanate) ("HMDI") or hexamethylenediisocyanate ("HDI"). After

purification the resultant PEG diisocyanate is added dropwise to excess glycerol or

trimethylol propane or other triol and reacted to completion. The purified product, now

5   having diol end groups, is again reacted with excess diisocyanate and purified, yielding a

PEG-tetra-isocyanate. This tetrafunctional PEG subsequently may be reacted with excess

PEG diols, yielding a 4 arm PEG synthesized from a PEG diol oligomer. In the final step

lysine end groups are incorporated, as discussed previously.

Structure T may be synthesized as follows: First synthesize a random copolymer

10   of PEG-monoacrylate and some other acrylate or combination of acrylates, such that the

final polyacrylate is water soluble. Other acrylates include, but are not limited to, 2-

hydroxyethylacrylate, acrylic acid, and acrylamide. Conditions may be varied to control

the molecular weight as desired. In the final step, the acrylate is reacted with lysine as

discussed previously, using an appropriate quantity to achieve the desired degree of

15   amination.

One method of synthesizing Structures U-Y is to use dicyclohexylcarbodiimide

coupling to a carboxylate end group. For Structures U-W, one can react the appropriate

PEG-diol, -triol or -tetra-hydroxy starting material with excess succinic anhydride or

glutaric anhydride such that all end groups are effectively carboxylated. Structures X and

20   Y may be made in a manner similar to that used for Structures S and T, except that in the

last step instead of end capping with lysine, end capping with succinic anhydride or

glutaric anhydride is performed.

Preparation of Biocompatible Polymers

Several biocompatible crosslinked hydrogels may be produced using the

25   crosslinkers and functional polymers described in FIGS. 1 to 5. Preferred combinations of

such polymers suitable for producing such biocompatible crosslinked polymers are

5   described in Table 2. In Table 2, the crosslinker functional groups are N-hydroxy succinimide esters and the functional polymer functional groups are primary amines.

Table 2.Biocompatible Polymers Synthesized from Crosslinkers and Functional Polymers of Table 1

Crosslinker Structure	Functional Polymer Structure	Concentration	Medium
B or C	H and R	Molar Equivalent; > 20% W/V	Borate or triethanol amine buffer, pH 7-10
A, B or C	H, P, Q, R and S	Molar Equivalent; > 20% W/V	Borate or triethanol amine buffer, pH 7-10
Y	T, H, P and Q	Molar Equivalent; > 10% W/V	Borate or triethanol amine buffer, pH 7-910
W, V	H and J	Molar Equivalent; > 20% W/V	Bicarbonate buffer, pH 7-10
X	I, J and H	Molar Equivalent; > 20% W/V	Borate or triethanol amine buffer, pH 7-10

10   The reaction conditions for crosslinking will depend on the nature of the functional groups. Preferred reactions are conducted in buffered aqueous solutions at pH 5 to 12. The preferred buffers are sodium borate buffer (pH 10) and triethanol amine buffer (pH 7). Elevated pH increases the speed of electrophilic-nucleophilic reactions.

15   In some embodiments, organic solvents such as ethanol or isopropanol may be added to improve the reaction speed or to adjust the viscosity of a given formulation.

The synthetic crosslinked gels described above degrade due to hydrolysis of the biodegradable region. The degradation of gels containing synthetic peptide sequences will depend on the specific enzyme and its concentration. In some cases, a specific

5   enzyme may be added during the crosslinking reaction to accelerate the degradation process.

When the crosslinker and functional polymers are synthetic (for example, when they are based on polyalkylene oxide), then it is desirable and in some cases essential to use molar equivalent quantities of the reactants. In some cases, molar excess crosslinker

10   may be added to compensate for side reactions such as reactions due to hydrolysis of the functional group.

When choosing the crosslinker and crosslinkable polymer, at least one of polymers must have more than 2 functional groups per molecule and at least one degradable region, if it is desired that the resultant biocompatible crosslinked polymer be

15   biodegradable. For example, the difunctional crosslinker shown as Structure A in FIG. 1 cannot form a crosslinked network with the difunctional polymers shown as Structure F in FIG. 2 or Structure P in Fig. 4. Generally, it is preferred that each biocompatible crosslinked polymer precursor have more than 2 and more preferably 4 or more functional groups.

20   Preferred electrophilic functional groups are NHS, SNHS and ENHS (FIG. 9). Preferred nucleophilic functional groups are primary amines. The advantage of the NHS-amine reaction is that the reaction kinetics lead to quick gelation usually within 10 about minutes, more usually within about 1 minute and most usually within about 10 seconds. This fast gelation is preferred for in situ reactions on live tissue.

25   The NHS-amine crosslinking reaction leads to formation of N-hydroxysuccinimide as a side product. The sulfonated or ethoxylated forms of N-hydroxysuccinimide are preferred due to their increased solubility in water and hence

5   their rapid clearance from the body. The sulfonic acid salt on the succinimide ring does

not alter the reactivity of NHS group with the primary amines.

The NHS-amine crosslinking reaction may be carried out in aqueous solutions

and in the presence of buffers. The preferred buffers are phosphate buffer (pH 5.0-7.5).

triethanolamine buffer (pH 7.5-9.0) and borate buffer (pH 9.0-12) and sodium

10   bicarbonate buffer (pH 9.0-10.0).

Aqueous solutions of NHS based crosslinkers and functional polymers preferably

are made just before the crosslinking reaction due to reaction of NHS groups with water.

Longer "pot life" may be obtained by keeping these solutions at lower pH (pH 4-5).

The crosslinking density of the resultant biocompatible crosslinked polymer is

15   controlled by the overall molecular weight of the crosslinker and functional polymer and

the number of functional groups available per molecule. A lower molecular weight

between crosslinks such as 600 will give much higher crosslinking density as compared

to a higher molecular weight such as 10,000. Higher molecular weight functional

polymers are preferred, preferably more than 3000 so as to obtain elastic gels.

20   The crosslinking density also may be controlled by the overall percent solids of

the crosslinker and functional polymer solutions. Increasing the percent solids increases

the probability that an electrophilic functional group will combine with a nucleophilic

functional group prior to inactivation by hydrolysis. Yet another method to control

crosslink density is by adjusting the stoichiometry of nucleophilic functional groups to

25   electrophilic functional groups. A one to one ratio leads to the highest crosslink density.

Preparation of Biodegradable Polymers

5   The biodegradable crosslinkers described in FIGS. 1 and 3 may be reacted with proteins, such as albumin, other serum proteins, or serum concentrates to generate crosslinked polymeric networks. Briefly, aqueous solutions of the crosslinkers described in FIG. 1 and FIG. 3 (at a concentration of 50 to 300 mg/ml) are mixed with concentrated solutions of albumin (600 mg/ml) to produce a crosslinked hydrogel. This reaction can be

10   accelerated if a buffering agent, e.g., borate buffer or triethanol amine, is added during the crosslinking step.

The resultant crosslinked hydrogel is a semisynthetic hydrogel whose degradation depends on the degradable segment in the crosslinker as well as degradation of albumin by enzymes. In the absence of any degradable enzymes, the crosslinked polymer will

15   degrade solely by the hydrolysis of the biodegradable segment. If polyglycolate is used as the biodegradable segment, the crosslinked polymer will degrade in 1-30 days depending on the crosslinking density of the network. Similarly, a polycaprolactone based crosslinked network will degrade in 1-8 months. The degradation time generally varies according to the type of degradable segment used, in the following order:

20   polyglycolate < polylactate < polytrimethylene carbonate < polycaprolactone. Thus it is possible to construct a hydrogel with a desired degradation profile, from a few days to months, using a proper degradable segment.

The hydrophobicity generated by biodegradable blocks such as oligohydroxy acid blocks or the hydrophobicity of PPO blocks in Pluronic or Tetronic polymers are helpful

25   in dissolving small organic drug molecules. Other properties which will be affected by incorporation of biodegradable or hydrophobic blocks are: water absorption, mechanical properties and thermosensitivity.

5   Methods of Using Biocompatible Polymers

The biocompatible crosslinked polymers and their precursors described above may be used in a variety of applications, such as components of tissue adhesives, tissue sealants, drug delivery vehicles, wound covering agents, barriers in preventing postoperative adhesions, and others. These and other suitable applications are reviewed in

10   Schlag and Redl, "Fibrin Sealant" in Operative Surgery, volumes 1-7 (1986), which is incorporated herein by reference.

In Situ Formation

In many applications, the biocompatible crosslinked polymers of this invention typically will be formed "in situ" at a surgical site in the body. The various

15   methodologies and devices for performing "in situ" gelation, developed for other adhesive or sealant systems such fibrin glue or sealant applications, may be used with the biocompatible crosslinked polymers of this invention. Thus, in one embodiment, an aqueous solution of a freshly prepared crosslinker (e.g., SNHS-terminated oligolactide synthesized from a glycerol core in phosphate buffered saline ("PBS") at pH 5 to 7.2) and

20   a functional polymer (e.g., albumin or amine terminated tetrafunctional polyethylene glycol at pH 10 in sodium borate) are applied and mixed on the tissue using a double barrel syringe (one syringe for each solution). The two solutions may be applied simultaneously or sequentially. In some embodiments, it is preferred to apply the precursor solutions sequentially so as to "prime" the tissue, resulting in improved

25   adherence of the biocompatible crosslinked polymer to the tissue. Where the tissue is primed, the crosslinker precursor is preferably applied to the tissue first, followed by the functional polymer solution.

5   One may use specialized devices to apply the precursor solutions, such as those described in U.S. Patent Nos. 4,874,368; 4,631,055; 4,735,616; 4,359,049; 4,978,336; 5,116,315; 4,902,281; 4,932,942; Published Patent Cooperation Treaty Patent Application No. WO 91/09641; and R.A. Tange, "Fibrin Sealant" in Operative Medicine: Otolaryngology, volume 1 (1986), the disclosures of which are herein incorporated by

10   reference.

Drug Delivery

The subject crosslinkers, functional polymer and their reaction products, the crosslinked materials advantageously may be used for localized drug therapy. Biologically active agents or drug compounds that may be added and delivered from the

15   crosslinked polymer or gel include: proteins, glycosaminoglycans, carbohydrates, nucleic acid, inorganic and organic biologically active compounds where specific biologically active agents include but are not limited to: enzymes, antibiotics, antineoplastic agents, local anesthetics, hormones, angiogenic agents, anti-angiogenic agents, growth factors, antibodies, neurotransmitters, psychoactive drugs, anticancer drugs, chemotherapeutic

20   drugs, drugs affecting reproductive organs, genes, and oligonucleotides.

To prepare such crosslinked composition, the bioactive compounds described above are mixed with the crosslinkable polymer prior to making the aqueous solution or during the aseptic manufacturing of the functional polymer. This mixture then is mixed with the crosslinker to produce a crosslinked material in which the biologically active

25   substance is entrapped. Functional polymers made from inert polymers like Pluronic, Tetronics or Tween" surfactants are preferred in releasing small molecule hydrophobic drugs.

5   In a preferred embodiment, the active agent or agents are present in a separate phase when crosslinker and crosslinkable polymers are reacted to produce a crosslinked polymer network or gel. This phase separation prevents participation of bioactive substance in the chemical crosslinking reaction such as reaction between NHS ester and amine group. The separate phase also helps to modulate the release kinetics of active

10   agent from the crosslinked material or gel, where 'separate phase' could be oil (oil-in water emulsion), biodegradable vehicle, and the like. Biodegradable vehicles in which the active agent may be present include: encapsulation vehicles, such as microparticles, microspheres, microbeads, micropellets, and the like, where the active agent is encapsulated in a bioerodable or biodegradable polymers such as polymers and

15   copolymers of: poly(anhydride), poly(hydroxy acid)s, poly(lactone)s, poly(trimethylene carbonate), poly(glycolic acid), poly(lactic acid), poly(glycolic acid)-co-poly(glycolic acid), poly(orthocarbonate), poly(caprolactone), crosslinked biodegradable hydrogel networks like fibrin glue or fibrin sealant, caging and entrapping molecules, like cyclodextrin, molecular sieves and the like. Microspheres made from polymers and

20   copolymers of poly(lactone)s and poly(hydroxy acid) are particularly preferred as biodegradable encapsulation vehicles.

In using crosslinked materials which are described herein as drug delivery vehicles, the active agent or encapsulated active agent may be present in solution or suspended form in crosslinker component or functional polymer solution component. The

25   nucleophilic component, whether it be in the crosslinker or the functional polymer is the preferred vehicle due to absence of reactive groups. The functional polymer along with bioactive agent, with or without encapsulating vehicle, is administered to the host along

5   with equivalent amount of crosslinker and aqueous buffers. The chemical reaction between crosslinker and the functional polymer solution readily takes place to form a crosslinked gel and acts as a depot for release of the active agent to the host. Such methods of drug delivery find use in both systemic and local administration of an active agent.

10   In using the crosslinked composition for drug delivery as mentioned above, the amount of crosslinkable polymer, crosslinker and the dosage agent introduced in the host will necessarily depend upon the particular drug and the condition to be treated. Administration may be by any convenient means such as syringe, canula, trocar, catheter and the like.

15   Several methods for the formation of regional adhesion barriers are described, in which any of a variety of water soluble macromeric precursors are used. The term "macromeric precursor" or "macromer" is meant to connote an oligomeric or polymeric molecule that contains functional groups that enable further crosslinking. Preferably the functionality of a macromer molecule is >2 so that a crosslinked network or hydrogel

20   results upon crosslinking.

In one embodiment, a crosslinked regional barrier is formed in situ, for example, by electrophilic-nucleophilic reaction, free radical polymerization initiated by a redox system or thermal initiation, wherein two components of an initiating system are simultaneously, sequentially or separately instilled in a body cavity to obtain widespread

25   dispersal and coating of all or most visceral organs within that cavity prior to gelation and crosslinking of the regional barrier. Once the barrier is formed, the organs remain

5   isolated from each other for a predetermined period, depending upon the absorption profile of the adhesion barrier material.

Preferably, the barrier is selected to have a low stress at break in tension or torsion, so as to not adversely affect normal physiological function of visceral organs within the region of application. The barrier also may contain a drug or other therapeutic

10   agent.

Certain embodiments of the invention are accomplished by providing compositions and methods to control the release of relatively low molecular weight therapeutic species using hydrogels. In accordance with the principles of the present invention, a therapeutic species first is dispersed or dissolved within one or more

15   relatively hydrophobic rate modifying agents to form a mixture. The mixture may be formed into microparticles, which are then entrapped within a bioabsorbable hydrogel matrix so as to release the water soluble therapeutic agents in a controlled fashion. Alternatively, the microparticles may be formed in situ during crosslinking of the hydrogel.

20   In one method of the present invention, hydrogel microspheres are formed from polymerizable macromers or monomers by dispersion of a polymerizable phase in a second immiscible phase, wherein the polymerizable phase contains at least one component required to initiate polymerization that leads to crosslinking and the immiscible bulk phase contains another component required to initiate crosslinking,

25   along with a phase transfer agent. Pre-formed microparticles containing the water soluble therapeutic agent may be dispersed in the polymerizable phase, or formed in situ, to form an emulsion. Polymerization and crosslinking of the emulsion and the

5 immiscible phase is initiated in a controlled fashion after dispersal of the polymerizable phase into appropriately sized microspheres, thus entrapping the microparticles in the hydrogel microspheres. Visualization agents may be included, for instance, in the microspheres, microparticles, and/or microdroplets.

Embodiments of the invention include compositions and methods for forming

10 composite hydrogel-based matrices and microspheres having entrapped therapeutic compounds. In one embodiment, a bioactive agent is entrapped in microparticles having a hydrophobic nature (herein called "hydrophobic microdomains"), to retard leakage of the entrapped agent. More preferably, the composite materials that have two phase dispersions, where both phases are absorbable, but are not miscible. For example, the

15 continuous phase may be a hydrophilic network (such as a hydrogel, which may or may not be crosslinked) while the dispersed phase may be hydrophobic (such as an oil, fat, fatty acid, wax, fluorocarbon, or other synthetic or natural water immiscible phase, generically referred to herein as an "oil" or "hydrophobic" phase).

The oil phase entraps the drug and provides a barrier to release by slow

20 partitioning of the drug into the hydrogel. The hydrogel phase in turn protects the oil from digestion by enzymes, such as lipases, and from dissolution by naturally occurring lipids and surfactants. The latter are expected to have only limited penetration into the hydrogel, for example, due to hydrophobicity, molecular weight, conformation, diffusion resistance, etc. In the case of a hydrophobic drug which has limited solubility in the

25 hydrogel matrix, the particulate form of the drug may also serve as the release rate modifying agent.

5   Hydrophobic microdomains, by themselves, may be degraded or quickly cleared when administered in vivo, making it difficult to achieve prolonged release directly using microdroplets or microparticles containing the entrapped agent in vivo. In accordance with the present invention, however, the hydrophobic microdomains are sequestered in a gel matrix. The gel matrix protects the hydrophobic microdomains from rapid clearance,

10   but does not impair the ability of the microdroplets or microparticles to release their contents slowly. Visualization agents may be included, for instance, in the gel matrix or the microdomains.

         In one embodiment, a microemulsion of a hydrophobic phase and an aqueous solution of a water soluble molecular compound, such as a protein, peptide or other water

15   soluble chemical is prepared. The emulsion is of the "water-in-oil" type (with oil as the continuous phase) as opposed to an "oil-in-water" system (where water is the continuous phase). Other aspects of drug delivery are found in commonly assigned U.S. patent applications 09/134,287 entitled "Composite Hydrogel Drug Delivery Systems"; 09/390,046 entitled "Methods and Apparatus for Intraluminal Deposition of Hydrogels";

20   and 09/134,748 entitled "Methods for Forming Regional Tissue Adherent Barriers and Drug Delivery Systems", each of which are hereby incorporated by reference.

         In another aspect of the present invention, the hydrogel microspheres are formed having a size that will provide selective deposition of the microspheres, or may linked with ligands that target specific regions or otherwise affect deposition of the

25   microspheres within a patient's body.

         Controlled rates of drug delivery also may be obtained with the system of the present invention by degradable, covalent attachment of the bioactive molecules to the

5 crosslinked hydrogel network. The nature of the covalent attachment can be controlled to enable control of the release rate from hours to weeks or longer. By using a composite made from linkages with a range of hydrolysis times, a controlled release profile may be extended for longer durations.

Composite Biomaterials

10 The biocompatible crosslinked polymers of this invention optionally may be reinforced with flexible or rigid fibers, fiber mesh, fiber cloth and the like. The insertion of fibers improves mechanical properties like flexibility, strength, and tear resistance. In implantable medical applications, biodegradable fibers, cloth, or sheets made from oxidized cellulose or poly(hydroxy acid)s polymers like polylactic acid or polyglycolic

15 acid, are preferred. Such reinforced structures may be produced using any convenient protocol known in the art.

In a preferred method, aqueous solutions of functional polymers and crosslinkers are mixed in appropriate buffers and proportions are added to a fiber cloth or net such as Interceed (Ethicon Inc., New Brunswick, NJ). The liquid mixture flows into the

20 interstices of the cloth and becomes crosslinked to produce a composite hydrogel. Care is taken to ensure that the fibers or fiber mesh are buried completely inside the crosslinked hydrogel material. The composite structure can be washed to remove side products such as N-hydroxysuccinimide. The fibers used are preferably hydrophilic in nature to ensure complete wetting of the fibers by the aqueous gelling composition.

25 EXAMPLES

The following non-limiting examples are intended to illustrate the synthesis of new biocompatible crosslinked polymers and their precursors, and their use in making

5   several medical products. Those skilled in the art will appreciate that modifications can

be made to these examples, drawings, illustrations and claims that are intended to fall

within the scope of the present invention.

Materials and Equipment

Polyethylene glycol was purchased from various sources such as Shearwater

10   Polymers, Union Carbide, Fluka and Polysciences. Multifunctional hydroxyl and amine

terminated polyethylene glycol were purchased from Shearwater Polymers, Dow

Chemicals and Texaco. Pluronic® and Tetronic® series polyols were purchased from

BASF Corporation. DL-lactide, glycolide, caprolactone and trimethylene carbonate was

obtained from commercial sources like Purac, DuPont, Polysciences, Aldrich, Fluka,

15   Medisorb, Wako and Boehringer Ingelheim. N-hydroxysulfosuccinimide was purchased

from Pierce. All other reagents, solvents were of reagent grade and were purchased from

commercial sources such as Polysciences, Fluka, Aldrich and Sigma. Most of the

reagents and solvents were purified and dried using standard laboratory procedures such

as described in D.D. Perrin et al., Purification of Laboratory Chemicals (Pergamon Press

20   1980).

General Analysis

The polymers synthesized according to these examples were chemically analyzed

using structure-determining methods such as nuclear (proton and carbon-13) magnetic

resonance spectroscopy, infrared spectroscopy. Molecular weights were determined using

25   high pressure liquid chromatography and gel permeation chromatography. Thermal

characterization of the polymers, including melting point and glass transition

temperatures, were performed using differential scanning calorimetric analysis. Aqueous

5   solution properties such as micelle and gel formation was determined using fluorescence spectroscopy, UV-visible spectroscopy and laser light scattering instruments.

In vitro degradation of the polymers was followed gravimetrically at 37°C, in an aqueous buffered medium such as phosphate buffered saline (at pH 7.2). In vivo biocompatibility and degradation life times was assessed by injecting or forming a gelling

10   formulation directly into the peritoneal cavity of a rat or rabbit and observing its degradation over a period of 2 days to 12 months.

Alternatively, the degradation was also assessed by prefabricating a sterile implant, made by a process like solution casting, then surgically implanting the implant within an animal body. The degradation of the implant over time was monitored

15   gravimetrically or by chemical analysis. The biocompatibility of the implant was assessed by standard histological techniques.

Example 1. Synthesis of a water-soluble difunctional, biodegradable functional polymer based on polyalkylene oxide block copolymer.

20   First, Polyethylene glycol-co-polycaprolactone polyol ("F68C2") was synthesized as follows:

30g of Pluronic F68 was dried under vacuum at110°C for 6 h and then mixed with 1.710 g of caprolactone and 30 mg of stannous 2-ethylhexanoate in a glass sealing tube. The glass tube then was sealed under nitrogen atmosphere and heated to 170°C and

25   maintained at this temperature for 16 h. The Pluronic F68-caprolactone polymer was cooled and recovered by breaking the glass sealing tube, and then further purified by several precipitations from a toluene-hexane solvent-nonsolvent system.

5   The polymer then was dried in vacuum at 40°C and used immediately in the

activation reaction described below:

Reaction with succinic anhydride ("11F68C2S"):

30 g of Pluronic F68-caprolactone copolymer was dissolved in 200 ml dry N,N-

dimethyl formamide ("DMF") and 0.845 g of succinic anhydride was added to the

10   reaction mixture. The mixture was heated to 100°C under a nitrogen atmosphere for 16 h.

The solution then was cooled and added to 4000 ml hexane to precipitate the carboxyl

terminated polymer. It was further purified by repeated (3 times) precipitation from a

toluene-hexane solvent-nonsolvent system. The polymer was dried under vacuum at

40°C.

15   This polymer was immediately used in activation reaction described below:

Activation of carboxyl groups with N-hydroxysuccinimide ("F68C2SSNHS"):

30 g of Pluronic F68-caprolactone succinate copolymer was dissolved in 200 ml

dry DMF. The solution was cooled to 4°C and 1.504 g of 1,3-dicyclohexylcarbodiimide

("DCC"I) and 1.583 g of N-hydroxysulfosuccinimide ("SNHS") were added to the

20   reaction mixture. The mixture was stirred at 4°C for 6 h and then stirred overnight at

room temperature under nitrogen atmosphere. Dicyclohexylurea was removed by

filtration and the F68C2S-SNHS derivative was isolated by removing the DMF under

vacuum and repeated precipitation using a toluene-hexane solvent-nonsolvent system.

The product was stored under nitrogen atmosphere at -20°C.

25   **Example 2.** Amine terminated synthetic biodegradable crosslinkable polymer.

Reaction of F68TMC2SSNHS with Lysine.

5   3.55 g of lysine was dissolved in 200 ml 0.1M borate buffer (pH 8.5). The

mixture was cooled to 0°C in ice bath and 10 g of F68C2SSNHS were added to the

mixture. The mixture was stirred for 6 h at room temperature and lyophilized. The

lyophilized powder was dissolved in 30 ml toluene and filtered. The filtrate was added to

4000 ml cold diethyl ether. The precipitated amine terminated polymer was recovered by

10   filtration and dried under vacuum. The polymer was stored under argon at -20°C.

Example 3. Synthesis of carboxyl terminated oligolactic acid polymer activated with N-

hydroxysulfosuccinimide.

Synthesis of difunctional oligolactate with terminal carboxyl acid end-groups

activated with N-hydroxysulfosuccinimide groups.

15   Part 1: Synthesis of oligomeric poly(lactic acid) with terminal carboxyl acid

groups ("PLA-S").

In a 250 ml 3 neck flask equipped with mechanical stirrer, nitrogen inlet and

distillation condenser, 2 grams of succinic acid and 34.1 ml 1N HC 1and 3.83 g L-lactic

acid, sodium salt were charged. The flask was then immersed in a silicone oil bath

20   maintained at 150°C. Most of the water from the reaction mixture was removed over

period of 5 hours by distillation. The remaining water was removed by heating the

reaction mixture under vacuum at 180°C for 15 h. The reaction mixture was cooled and

1yophilized at 0°C to remove traces of water. The product was isolated by dissolving in

toluene and precipitating in hexane. The precipitated polymer was isolated by filtration

25   and dried in vacuum for 48 h at 60°C.

5   Part 2: Activation of terminal groups with N-hydroxysulfosuccinimide group:

A 3 necked flask equipped with magnetic stirrer and nitrogen inlet was charged

with 2 g of PLA-S copolymer and 20 ml DMF. The solution was cooled 4°C and 3.657 g

of N-hydroxysulfosuccinimide and 3.657 g of 1,3-dicyclohexyl carbodiimide were added

to the reaction mixture. The mixture was stirred at 4°C for 6 h and overnight at room

10   temperature under nitrogen atmosphere. Dicyclohexylurea was removed by filtration and

SNHS derivative was by isolated by removing the DMF under vacuum and repeated

precipitation using toluene-hexane solvent-nonsolvent system. The product was stored

under nitrogen atmosphere at 4°C.

15   **Example 4.** Preparation of polyethylene glycol based tetrafunctional crosslinker.

Part 1: Synthesis of tetrafunctional polyethylene glycol-co-polyglycolate
copolymer ("4PEG2KG"):

30 grams of 4 arm polyethylene glycol, molecular weight 2000 ("4PEG2K") was

dried at 100°C for 16 hours prior to use. 30 grams 4PEG2K. 7.66 g of glycolide and 25

20   mg of stannous 2-ethylhexanoate were charged into a 3 necked flask equipped with a

Teflon coated magnetic stirring needle. The flask was then immersed into silicone oil

bath maintained at 160°C. The polymerization reaction was carried out for 16 h under

nitrogen atmosphere. At the end of the reaction, the reaction mixture was dissolved in

100 ml toluene. The hydroxy terminated glycolate copolymer was isolated by pouring

25   the toluene solution in 4000 ml cold hexane. It was further purified by repeated

5   dissolution-precipitation process from toluene-hexane solvent-nonsolvent system and

dried under vacuum at 60°C. It then was immediately used for end capping reaction

mentioned below:

Part 2: Conversion of hydroxyl groups into carboxylic groups ("4PEG2KGS")

and SNHS ester.

10   30 g of 4PEG2KG copolymer was dissolved in 150 ml dry pyridine. 8.72 g of

succinic anhydride was added to it and the solution was refluxed for 2 h under nitrogen

atmosphere. The polymer was isolated by pouring the cold pyridine solution to 4000 ml

hexane. The acid terminated polymer ("4PEG2KGS") was used in SNHS activation

reaction. Briefly, to a solution of 30 g of 4PEG2KGS in 300 ml dry methylene chloride

15   were added 10.58 g of SNHS and 10.05 g DCC. The reaction mixture was stirred

overnight under nitrogen atmosphere. Dicyclohexylurea was removed by filtration. The

filtrate was evaporated and the residue obtained was redissolved in 100 ml toluene. The

toluene solution was precipitated in 2000 ml hexane. The SNHS activated polymer was

stored under nitrogen atmosphere until further use.

20   **Example 5.** Sulfonyl chloride activated crosslinkers.

Activation of tetrafunctional polyethylene glycol-co-polyglycolate copolymer

("4PEG2KGS") with tresyl chloride.

30 g of 4PEG2KG was dissolved in 10 ml dry benzene. The solution was cooled

to O°C and 5.92 g of triethyl amine and 10.70 g tresyl chloride were added under

25   nitrogen atmosphere. After refluxing for 3 h under nitrogen atmosphere, the reaction

mixture was cooled and filtered to remove triethylamine hydrochloride. The filtrate was

5 poured into 3000 ml hexane to precipitate the activated polymer. The residue was redissolved in THF and filtered over neutral alumina to remove traces of triethylamine hydrochloride. The polymer was recovered by adding the THF solution to 3000 ml diethyl ether and stored under nitrogen atmosphere.

Example 6. Synthesis of multifunctional oligopolycaprolactone terminated with SNHS.

10 <u>Part 1: Synthesis of polycaprolactone ("PCL1").</u>

2.00 g of glycerol, 8.17 g of caprolactone and 50 mg of stannous 2-ethylhexanoate were charged into 100 ml Pyrex pressure sealing tube. The tube was frozen in liquid nitrogen and connected to vacuum line for 10 minutes. The tube then was connected to argon gas line and sealed under argon. The sealed reaction mixture then was immersed in

15 oil bath maintained at 160°C and polymerization was carried out for 16 h at 160°C. The polymer was recovered by dissolving it in 30 ml toluene and precipitating in 2000 ml cold hexane. The precipitated liquid oligomer was recovered and dried under vacuum for 1 day at 60°C.

<u>Part 2: End-capping of PCL1 with succinic anhydride ("PCL-S"):</u>

20 10 g of PCL1 was dissolved in 150 ml dry benzene. About 50 ml of benzene was distilled to remove traces of water from the reaction mixture. The solution was cooled to 30°C. To this warm solution, 6.67 g of triethyl amine and 7.86 g of succinic anhydride were added. The reaction mixture was then refluxed for 6 h and concentrated by distillation under vacuum. The product was recovered by adding the filtrate to 2000 ml

25 cold dry hexane.

<u>Part 3: Activation of PCL-S with SNHS:</u>

5   PCL1-succinate (5.0 g) was dissolved in 10 ml of anhydrous methylene chloride,

cooled to O°C and 7.82 g of N-hydroxysulfosuccinimide and 7.42 N, N-

dicyclohexylcarbodiimide were added under stirring.   After stirring the mixture

overnight, the precipitated dicyclohexylurea was removed by filtration and the solution

was concentrated by removing solvent. The ^{1}H-NMR spectrum showed succinimide

10   singlet at 2.80 ppm (2H).

Example 7. Preparation of polyethylene glycol-co-polytrimethylene carbonate copolymer

terminated with N-hydroxysuccinimide.

Preparation of tetrafunctional polyethylene glycol-co-polytrimethylene carbonate

copolymer ("4PEG10KTMC2").

15   30 g of tetrahydroxy polyethylene glycol, molecular weight 10000, was dried

under vacuum at 90-100°C in a glass sealing tube. The tube then was cooled and

transferred inside an air bag where 2.45 g of trimethylene carbonate and 20 mg of

stannous octoate were added to the tube. The glass tube was then sealed under vacuum

and heated with stirring at 155°C and maintained at this temperature for 16 h. The

20   polyethylene glycol-co-polytrimethylene carbonate polymer was cooled and recovered by

breaking the glass sealing tube. It was further purified by several precipitations from

toluene-hexane solvent-nonsolvent system.

Part   2:   Synthesis   of   glutarate   derivative   of   4PEG10KTMC2

("4PEG10KTMC2G"):

25   10 g of 4PEG10KTMC was dissolved in 120 ml dry toluene. About 50 ml of

toluene was distilled to remove traces of water from the reaction mixture. The warm

5   solution was cooled to 60°C. To this solution, 1.23 g of triethyl amine and 1.40 g of

glutaric anhydride were added. The reaction mixture was heated to 60°C for 1 h and

filtered. The product was recovered by adding the filtrate to 2000 ml cold dry hexane.

Part 3: Activation of terminal carboxyl groups using N-hydroxysuccinimide

("4PEG10KTMC2GNHS"):

10   30 g of 4PEG10KTMC2G was dissolved in 100 ml of dry DMF and 1.53 g of N-

hydroxysuccinimide and 5 g molecular sieves 3Å were added. 1.28 g of DCC dissolved

in 5 ml dry DMF was added dropwise and the reaction mixture was kept at room

temperature for 24 h under nitrogen atmosphere. The mixture was diluted with 50 ml cold

benzene and precipitated using cold hexane. The precipitate was collected on a sintered

15   glass filter with suction. The dissolution and precipitation procedure was then repeated

three times, using toluene-diethyl ether as solvent-nonsolvent system and dried under

vacuum. The product was stored under nitrogen atmosphere at −20°C until further use.

Example 8. Succinated polyhydroxy compounds activated with N-hydroxysulfosuccinimide ES.

20   10 g of erythritol was dissolved in 200 ml dry toluene. About 50 ml of toluene

was distilled to remove traces of water from the erythritol. The solution was cooled to 50-

60°C and 20 ml pyridine and 8.58 g of succinic anhydride were added to the solution.

The reaction mixture was then refluxed for 3 h and unreacted pyridine and toluene were

evaporated to dryness under reduced pressure. The residue was used in activation

25   reaction.

5   <u>Part 2: Activation of ES with SNHS:</u>

Erythritol-succinate (ES, 2.0 g) was dissolved in 10 ml of anhydrous dimethyl formamide ("DMF"), cooled to O°C and 3.47 g of N-hydroxysulfosuccinimide and 3.30 N, N-dicyclohexylcarbodiimide were added under stirring. After stirring the mixture overnight, the precipitated dicyclohexylurea was removed by filtration and the solution

10   was concentrated by removing solvent. It was further purified by column chromatography.

Example 9. Preparation of synthetic crosslinked biodegradable gels.

1.57 g (0.8 mM) of 4 arm amine terminated polyethylene glycol molecular weight 2000 was dissolved in 10 ml 0.1 M sodium borate buffer at pH 9.5 2 g of 4 arm SNHS

15   activated 4PEG2KGS polymer (molecular weight 2500) was dissolved in phosphate buffered saline. These two solutions were mixed to produce a crosslinked gel. In another variation of this method, the 4PEG2KGS polymer solid was directly added to the amine terminated polymer solution to produce a crosslinked polymer.

In another variation, a crosslinker consisting of an equimolar solution of dilysine

20   can be used in place of the 4 arm PEG amine solution to form a hydrogel. Gelation was seen to occur within 10 seconds of mixing the two solutions. Similarly, other crosslinkers described in examples 1 to 7 may be reacted in molar equivalent proportions with other amine terminated polymers such as albumin or amine terminated biodegradable polymers similar to described in Example 2. The preferred compositions for making biodegradable

25   hydrogels were described in Table 2. The amine terminated polymer solution described above was added with 0.1% of F D and C blue or indigo dye prior to crosslinking reaction. The addition of dye allows the preparation of colored gels.

5   **Example 10.** Preparation of composite synthetic crosslinked colored biodegradable gels.

3 grams of bovine serum albumin was dissolved in 3 ml of phosphate buffered solution. Commercial sutures based on synthetic biodegradable polymers, such as Vicryl was cut/ground into several small pieces (size less than 1 mm) using cryogenic grinding. These colored suture particles (approximately 100 mg) were mixed with the albumin

10   solution to form a suspension. 100 mg of crosslinker such as 4PEG10KTMC2GNHS was mixed with 0.2 ml of albumin suspension. This viscous solution then was mixed with 40 mg of triethanol amine (buffering agent). The addition of triethanol amine gels the solution in 60 seconds. The colored suture particles entrapped in the crosslinked gel help to visualize the gel especially when under laparoscopic conditions and also acts to

15   strengthen the hydrogel as a reinforcing agent. The suture particles in above examples can be replaced with biodegradable microparticles loaded with drugs or bioactive compounds.

Example 11. Formulation of SG-PEG with Di-lysine.

A four arm PEG with SG end groups (Shearwater Polymers, approx. 9,100 g/mol,

20   0.704 grams, 6.5×10^{-5} moles) was dissolved in 2.96 g 0.01M pH 4.0 phosphate buffer (19.2% solids). Di-lysine (Sigma, 347.3 g/mol, 0.03 grams, 8.7×10^{-5} moles) was dissolved in 3.64 grams of 0.1M pH 9.5 borate buffer (0.8% solids). On combination of the two solutions, the percent solids was 10%. The di-lysine has 3 amine groups. The SG-PEG has 4 NHS groups. After correction for the less than 100% degree of substitution on

25   the SG-PEG, the formulation gives a 1:1 stoichiometry of amine groups to NHS groups.

Example 12. Formulation of SG-PEG with Tri-lysine.

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5   A four arm PEG with SG end groups (Shearwater Polymers, approx. 9,100 g/mol, 0.675 grams, $6.2x10^{-5}$ moles) was dissolved in 2.82 g 0.01M pH 4.0 phosphate buffer (19.3% solids). Tri-lysine (Sigma, 402.5 g/mol, 0.025 grams, $6.2x10^{-5}$ moles) was dissolved in 3.47 grams of 0.1M pH 9.5 borate buffer (0.7% solids). On combination of the two solutions, the percent solids was 10%. The tri-lysine has 4 amine groups. The

10   SG-PEG has 4 NHS groups. After correction for the less than 100% degree of substitution on the SG-PEG, the formulation gives a 1:1 stoichiometry of amine groups to NHS groups.

Example 13. Formulation of SG-PEG with Tetra-lysine.

A four arm PEG with SG end groups (Shearwater Polymers, approx. 9,100 g/mol,

15   0.640 grams, $5.9x10^{-5}$ moles) was dissolved in 2.68 g 0.01M pH 4.0 phosphate buffer (19.2% solids). Tetra-lysine (Sigma, 530.7 g/mol, 0.025 grams, 4.7xlO-' moles) was dissolved in 3.30 grams of 0.1M pH 9.5 borate buffer (0.8% solids). On combination of the two solutions, the percent solids was 10%. The tetra-lysine has 5 amine groups. The SG-PEG has 4 NHS groups. After correction for the less than 100% degree of

20   substitution on the SG-PEG, the formulation gives a 1:1 stoichiometry of amine groups to NHS groups.

Example 14. Gel Time Measurement.

The amine solution (100 μL) was aliquotted into a 100x13 test tube. A flea-stirbar (7x2 mm, Fisher Scientific p/n 58948-976) was placed in the test tube. The test tube was

25   held stationary over a digital magnetic stirrer (VWR Series 400S Stirrer) set at 300 rpm. A 1 cc tuberculin syringe (Becton Dickinson, p/n BD309602) was filled with 100 μL of the ester solution. The syringe was inserted up to the flanges so that the distal end was

5   just over the amine solution. Simultaneously the plunger was depressed and a stop watch started. When the solution solidifies sufficiently so that the stir bar stops spinning, the stop watch was stopped. Each solution was measured in triplicate and the mean ±1 standard deviation was plotted. Results for the formulations of examples 1, 2 and 3 are shown in FIG. 11.

10   **Example 15.** Change in gel time as a function of ester solution age.

An important characteristic of these systems is the loss in reactivity over time from reconstitution of the ester solution. This loss in reactivity occurs due to hydrolysis of the N-hydroxysuccinimidyl ester, before the activated molecule can combine with its respective nucleophilic functional group. The loss of reactivity was characterized by

15   measuring the change in gel time as a function of time from reconstitution of the NHS ester solution. The gel time was measured periodically. The NHS ester solution was stored at ambient conditions during this measurement. Results for the solutions described in Examples 11, 12 and 13 are shown in FIG. 12.

Example 16. Gel formation at different percent solids from 4 arm CM-HBA-NS PEG

20   and Lys-Lys.

Using the gel time method described in Example 13, five different gel compositions were made using carboxymethyl hydroxybutyrate-hydroxysuccinimide end-capped 4 arm PEG (CM-HBA) (Shearwater Polymers) and di-lysine (Sigma). The formulations are listed below in Table 3.

25

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Table 3

Conc. (%)	CM-HBA (g)	Phosphate (g)	Lys-Lys (g)	Borate (g)
8.5	0.2469	1.264	0.01	1.5012
10	0.2904	1.2209	0.012	1.4994
12.5	0.363	1.1483	0.015	1.4964
15	0.4356	1.0757	0.018	1.4936
20	0.5808	0.9305	0.024	1.4876

The formulations were adjusted to give a 1 to 1 ratio of electrophilic functional end groups on the CM-HBA (4) to nucleophilic reactive groups on the di-lysine ("Lys-Lys")(3). The CM-HBA quantities were dissolved in 0.01M pH 5.0 phosphate buffer.

10 The di-lysine was dissolved in 0.1M pH 11 borate buffer. Gel time results are shown in Figure 13. This data also shows that the higher percent solids solutions also are the most stable with respect to retention of speed of reaction.

Example 17. Degradation of Hydrogels.

15 Hydrogel plugs made during the gel time measurements of Example 14 were placed in approximately 25 mL 0.1M phosphate buffered saline at pH 7.4 in 50 mL Falcon tubes and placed in a constant temperature bath at 37°C. The hydrogel plugs were observed visually at periodic intervals and the time of gel disappearance noted. The data are plotted in Figure 14.

20 **Example 18**. Precursor Spray Procedure to form a 7.5% solids hydrogel from 4 arm SG and dilysine.

An ethylene oxide sterilized air assisted sprayer was used in conjunction with aqueous solutions of polymerizable monomers. Solution 1 consisted of a 14.4% solution

5 of 4 arm SG (MW 10,000 purchased from Shearwater Polymers) dissolved in 0.01M

phosphate buffer at pH 4.0 and was sterile filtered (Pall Gelman syringe filter, p/n 4905)

and drawn up in a sterile 5 cc syringe. Solution 2 consisted of a 1.2% solution of a

dilysine (purchased from Sigma Chemicals) dissolved in 0.1M borate buffer at pH 11

with 0.5 mg/mL methylene blue for visualization and was also sterile filtered and drawn

10 up in a sterile 5 cc syringe. These solutions, when combined 1:1 on a volumetric basis,

resulted in a 1:1 ratio of NHS ester to amine end group. The final % solids after

combination was 7.5%. The two syringes were individually loaded in the two separate

receptacles through a luer-lok type of linkage. Airflow from a regulated source of

compressed air (an air compressor such as those commercially available for airbrushes)

15 was connected to the device using a piece of Tygon tube. On compressing the syringe

plungers a steady spray of the two liquid components was observed. When this spray was

directed to a piece of tissue (rat cecum) a hydrogel coating was observed to form on the

surface of the tissue. This hydrogel coating was rinsed with saline (the hydrogel coating

is resistant to rinsing) and was observed to be well adherent to the tissue surface. Within

20 a short period of time (less than a minute) an area of 10 cm X 5 cm could be coated with

ease.

Example 19. Precursor Spray Procedure to form a 12.5% solids hydrogel from 4 arm CM

and dilysine.

A hydrogel barrier film made from 4 arm CM-HBA NS (MW 10,000 purchased

25 from Shearwater Polymers), and dilysine was similarly prepared and sprayed as described

in Example 18. In the present example the 4 arm CM solution was made up to 24.0%

solids and the dilysine solution was made up to 1.0% solids such that on combination in

5　an equal volume delivery system a 1:1 ratio of NHS to amine end groups results, giving a final % solids of 12.5%.

Example 20. Spray Application of crosslinker and polymer to from crosslinked film.

Two solutions (component A and component B) were prepared. Component A consisted of dilysine in 0.1M borate buffer, pH 9.5. Component B consisted of either 4

10　arm SG-PEG or 4 arm CM-HBA-NS in 0.01M phosphate buffer, pH 4.0 These solutions were prepared such that the amine to ester stoichiometric ratio was 1:1 and the final total solution concentration was 7.5% or 12.5%, respectively.

A Fibriject™ (Micromedics, Inc.) 5 cc syringe holder and cap was used, preloaded with 5 cc of each solution and attached to a dual barrel atomizing sprayer. The

15　sprayer has two hubs for the syringes to connect to allowing the two fluids to be advanced through two separate lumens over any preset distance. A third hub exists for the application of the atomizing gas. Air was used in this example. The distal tip of the sprayer contains a chamber where the gas expands out of an introduction tube, then flows past the two polymer solution nozzles in an annular space around each. The gas is

20　accelerated in the annular spaces using a flow rate suitable for the complete atomization of the two fluid streams (~2L/min.). Two overlapping spray cones are thus formed allowing for well mixed, thin, uniform coatings to be applied to surfaces.

Example 21. Adhesion Prevention in Rat Cecum Model.

Surgical procedure

25　Male Sprague Dawley rats (250-300 grams,) were anesthetized with an intramuscular 4ml/kg "cocktail" of Ketamine (25 mg/ml), Xylazine (1.3mg/mL) and Acepromazine (0.33 mg/mL). The abdominal area was shaved and prepped for aseptic

5   surgery. A midline incision was made to expose the abdominal contents. The cecum was identified and location within the abdomen was noted. The cecum was pulled out of the abdomen and the surface of one side was abraded using dry sterile gauze. A technique of abrading one area by stroking the surface 12 times with the gauze was used. The cecal arterial supply was interrupted using bipolar coagulation along the entire surface area of

10   the damaged cecum.

The opposing abdominal sidewall which lays in proximity to the damaged cecal surface was deperitonealized with a scalpel blade and the underlying muscle layer was scraped to the point of hemorrhaging.

The cecum was sprayed with either the SG-PEG system or the CM-HBA-NS

15   system using the air assisted spray method described in the preceding example. The cecum was placed with the damaged (ischemic area) side up opposite the damaged side wall. Active bleeding was controlled before closing. The peritoneum and muscle wall was closed with 3-0 nylon and the skin was closed with 4-0 silk. Rats were returned to their cages for one to two weeks at which time evaluation of the adhesion between the

20   side wall and cecum was noted. The rats were killed at 10 days and the tenacity and extent of adhesion was evaluated. The results are summarized in Table 4.

Table 4

Rat #	Material Applied	Reference Example	Finding on Day 10
403	7.5% 4aSG with Lys-Lys w/MB	Example 18	Small amount of gel present on cecum. No adhesions from cecum to sidewall. No gel on sidewall
404	7.5% 4aSG with Lys-Lys w/MB	Example 18	Some mesentery stuck to cecum. No gel. No adhesions.
405	7.5% 4aSG with Lys-Lys	Example 18	Small amount of gel present on cecum. Some mesentery stuck to cecum and

	w/MB		sidewall. Some gel between mesentery and cecum where stuck. No adhesions.
406	12.5% 4aCM with Lys-Lys w/MB	Example 19	No gel present. No adhesions.
407	12.5% 4aCM with Lys-Lys w/MB	Example 19	No gel on cecum or sidewall. No adhesions.
408	12.5% 4aCM with Lys-Lys w/MB	Example 19	Rat died post-op (anesthesia overdose).

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Example 22

     This example is directed to concentrations of coloring agent for use in an in situ

crosslinked hydrogel coating. A 140 mg amount of four-arm primary amine terminated

10   polyethylene glycol molecule with a molecular weight of approximately 10,000 was

dissolved in sodium borate buffer pH 9.5. An 84 mg amount of four arm NHS activated

polyethylene glycol polymer (SPA-3400, Shearwater Corp., Huntsville, Al, molecular

weight approximately 3400) was dissolved in pH 5.0 acetate buffer. Methylene Blue was

added to the borate buffered solutions at concentrations of 0.1, 0.5, and 1.0 mg/ml.

15   A standard laparoscopic sprayer was used in a laparoscopic trainer to spray the

surfaces of pieces of lunch meat with an approximately 1:1 mixture of the solutions. The

mixture formed a gel in about 3-6 seconds on the surfaces. The sprayed gel was observed

through a 10 mm laparoscope and videotaped. The tapes were reviewed to assess the

effect of the coloring agent. The 0.5 mg/ml and 1.0 mg/ml solutions of coloring agent

20   created a gel that was readily observable and similar in visibility. The 0.1 mg/ml solution

of coloring agent created a gel that was light in color and more difficult to observe

compared to the other solutions. Many previous experiments had already shown that gels

5   with no coloring agents were very difficult to observe visually. Control experiments performed without the presence of methylene blue showed that the methylene blue did not affect gel times under these conditions.

A similar experiment was performed using 4 arm NHS polyethylene glycol (molecular weight 10,000) mixed with an equimolar concentration of a multiarm amine-

10   terminated polyethylene glycol (molecular weight 20,000). FD&C Blue #2 dye was present in the resultant hydrogel at a concentration of 0.05, 0.1, 0.25, 0.5, 1.0, and 2.5 mg/ml. The hydrogel was applied at a thickness of about 1.0 mm and observed by two independent observers and the ability to observe the gel was rated as adequate or inadequate. The results showed that good visualization of the hydrogel could be obtained

15   at concentrations of at least 0.25 mg/ml FD&C Blue #2.

Example 23

This example was directed to the evaluation of the stability of colorants in solution. An 0.5 mg/ml amount of FD&C BLUE #2 dye (also called indigo carmine) was dissolved in 0.1 M sodium borate decahydrate pH 10 buffer, in deionized water, and in

20   0.01 M sodium phosphate buffer pH 4.0. Solutions were stored for up to 48 hours at 4°C, 25°C, and 40°C. The dye appeared visually to be stable in solubility and color in the distilled water and phosphate buffer solutions although the dye changed the pH of the phosphate buffer from 4 to 6.9. The dye changed color in the borate solution. FD&C blue #2 and Methylene blue were observed to be not completely soluble so that their

25   concentration is significant. FD&C Blue #2 was soluble at less than 2.5 mg/ml but its maximum solubility was not ascertained.

5   Example 24

This experiment was directed to the effect of coloring agents on gelation times. A 4 arm NHS polyethylene glycol (molecular weight 10,000) solution was mixed with an equimolar concentration of a multiarm amine. Both an amine terminated polyethylene glycol (molecular weight 20,000) was evaluated as well as dilysine. Visualization agent was mixed with the buffer used to reconstitute the amine and was present in the resultant hydrogel at a concentration of 12.5 mg/ml. Gel time tests were performed in triplicate. Gel time was measured immediately on reconstitution of the ester (time zero) and 1.5 hours later. The mean gelation times in seconds ± standard deviation were: FD&C Blue #1 gelation time 1.57±0.12 time zero compared to 2.2±0.05 after 1.5 hours; FD&C Blue #2 gelation time 1.51±0.12 time zero compared to 2.08±0.09 after 1.5 hours; Methylene Blue gelation time 1.67±0.28 at time zero compared to 1.97±0.12 after 1.5 hours; No visualization agent gelation time 1.39±0.02 compared to 1.78 ±0.13 after 1.5 hours. These visualization agents did not cause an unacceptable change in gelation times.

20   While preferred illustrative embodiments of the invention are described above, it will be apparent to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is intended in the appended claims to cover all such changes and modifications which fall within the true spirit and scope of the invention.

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